

Hope at Last For European Collaboration

THE European Commission has produced a sensible plan for coordinating research and development, in basic science as well as in technology, within the enlarged community that will probably see the light of day in January (see page 476). The most laudable feature of the plan is the pragmatism which runs through it. And although the commission is as fierce as any other European organization on the need to make fuller use of European resources, both for the accomplishment of socially beneficial aims and economically valuable tasks, it now recognizes more plainly than ever that several years must pass before permanent institutions can emerge. This is a great step forward, for not so long ago, there was excited talk about the possibility of setting up a European Research and Development Agency to superintend and to finance technological projects organized on a community basis and to work on the framework, legal and administrative, within which European technology is carried out. In its latest plan, the commission says that its long-term goal will be the creation of some organization of this kind, but it has mercifully retreated from the position it took up at the Hague conference last year, when it appeared to be committed to the rapid setting up of an executive agency. Now it says that the first task is to discover "in the light of experience" how great should be the resources devoted to this kind of work and what kinds of management procedures and institutions should be set up.

The commission's proposals in basic research are especially welcome. Organizations such as CERN are held out as examples of how successful European collaboration can be, but the commission might have added that the difficulties of creating the European Molecular Biology Organization (EMBO) are a sign of how difficult the process of collaboration may be. So the commission is asking the Council of Ministers for authority to set up what is called a European Science Foundation. Sensibly enough, it recognizes that fundamental research in Europe "needs support rather than organization, stimulus rather than planning". The objects of the enterprise, which would be directed by a governing body of independent members, not national delegates, would be to foster the mobility of research workers, organizing international conferences, channelling funds to cooperative projects (such as CERN), picking out and then supporting laboratories which might become European centres of excellence (with the proviso that they must then admit researchers from elsewhere) and keeping a watch on national projects for building large and expensive instruments in case these should suggest opportunities for a pooling of resources. Especially if the foundation were to begin in a small way, by taking over responsibility for administering the funds which national governments at present spend on cooperative projects on a multinational basis, and if it had a modest sum to spend on fostering better communications within basic science, it could be doing useful work

quite soon. How soon it might flex its muscles and think of acting as a device for supporting research projects on a European basis, spending one member government's money in some other country, is more problematical.

The commission is properly more tentative in its proposals for coordinating industrial development. The past few years have seen a number of attempts at common planning and cooperative development, conspicuously the Aigrain working groups set up in 1964. The objective then was to identify some fields in which governments and industrial organizations might jointly undertake important pieces of technical development. The commission now recognizes that the outcome of the Aigrain proposals has been a disappointment. In some cases, as in computers, commercial interests and chauvinism between them have frustrated the plan for developing a European computer of mammoth size. The commission does not recognize as clearly as it should that other Aigrain projects—the exhortation to develop new means of transport, for example—were inadequately defined. Elsewhere, as in telecommunications, there has been a little progress in the outline planning of future needs, but there is still no prospect that the member nations of the European Community, all of whom are now working on the development of systems for the 1980s (see, for example, page 477), will reap the benefits that they might.

In the circumstances, it is sensible of the commission to recognize that the most important need is not for an organization but a talking shop. It suggests that there should be a European Research and Development Committee to give advice to the commission on research and development projects where opportunities for collaboration appear to exist. To begin with, it would be for the commission itself to undertake the formulation of specific proposals for common activities, for the Council of Ministers to give approval and then for the commission to finance by means of contracts at least some part of the collaborative work to be undertaken. As luck will have it, the commission now has a substantial body of experience in fields like these. The history of Euratom may be held to be enough to turn all but the most hot-blooded commissioners against the notion of European collaboration, but it remains a truth that several of the residual programmes now conducted cooperatively are a solid foundation on which to build. Yet the commission will have to move quickly if it is to make a useful contribution even in nuclear energy, where the opportunities are immense. On fast reactors, for example, the chances are that multilateral agreements between member states will undermine its influence. Plainly the new committee will have to work quickly but sensitively if it is to make a useful contribution.

Where should the new committee look for opportunities for collaboration, if in due course the Council of Ministers gives its blessing to the commission's plan?



And how is an organization which, by definition, will lack supra-national authority to hold the ring between governments, each seeking to get more benefit from collaboration than it contributes in resources, and industrial companies seeking (quite properly) to make quick profits and to put their competitors out of business? One kind of research worth sponsoring is that described by Dainton as strategic research—there is no reason why the European Commission should not think of sponsoring long-term technical development in, for example, the applications of solid state physics to electronics, so long as it is fairly understood that all interested governments and industrial companies should have an opportunity to learn from what goes on. Public service research, including medicine, is another field in which it may be possible for interested parties to subdue their wish to get more than a fair share from common expenditure. No harm will be done if the proposed committee on research and development were to make but a modest beginning in humdrum fields like these. But there are limits to what can be done on a European basis so long as national governments insist on behaving as if their first duty in the sponsorship of advanced technology is to improve the competitiveness of their national industries.

Nuclear Uncertainty

THE House of Commons Select Committee on Science and Technology has come to the right conclusion about the future of the non-nuclear research and development now carried out within the United Kingdom Atomic Energy Authority (see page 477). It was always intemperate of the Confederation of British Industry to have suggested that "it would make no difference whatever to industry" if the laboratory at Harwell, which has pioneered industrial contract research, were shut down. What the committee says is that Harwell is doing a useful job and this is entirely borne out by the evidence of the director of the laboratory, Dr Walter Marshall, to the committee on April 25. Since 1966, while the laboratory at Harwell has shrunk by 25 per cent, the amount of money collected in the form of payments for research and services has grown to 23 per cent of the laboratory's budget, and Dr Marshall estimates that by 1974, no less than 38 per cent of the laboratory's work will be supported in this way. Whatever the CBI may say, there appears to be no shortage of customers for the work which it is equipped to do. Indeed, given the doubts which inevitably attended the first beginnings of this attempt to turn a government laboratory into a commercially based research contracting agency, and the fear that the whole exercise was merely a fudge to keep the laboratory in being after it had outlived its usefulness, the committee might have been more fulsome in its congratulations. It could even reasonably have gone still further, and have pointed out the inconsistency with which the Department of Trade and Industry keeps urging that Harwell should find more work to do but only within the constraints of a planned decline of staff.

The Select Committee is however right to draw attention to the unfortunate consequences of continuing un-

certainty about the future of nuclear energy in Britain. Those engaged on non-nuclear work in the authority's laboratory are understandably unsettled when they hear the CBI sounding off and not in any way reassured by the continuing uncertainty about the future which is the Department of Trade and Industry's present substitute for a policy. But the uncertainty extends also to the future development of nuclear power stations in Britain. For several months, the DTI has been brooding on the Vinter report, supposedly a recommendation of what type of nuclear reactor should form the basis of the next decade's nuclear power programme in Britain. It would be no surprise if the outcome of this study was what might have been predicted in the first place—the difficulty of comparing the economics of different reactor types is so great, and the uncertainties are so many, that an unambiguous choice cannot be made. This, no doubt, is why Sir Douglas Allen, of the Treasury, is now at work on a scheme for reorganizing the nuclear industry. By all accounts, the questions to be decided are whether there will emerge a single public corporation embracing private industry and the Atomic Energy Authority and, if so, whether the controlling interest will be public or private. Evidently there are good reasons why the government cannot make up its mind about the next reactor until it has decided what shape the nuclear industry itself should take. At this late stage, no doubt, the DTI is well seized of the need for urgency. The puzzle is why it has dallied so long and why it has allowed so much damage to be done not merely to the morale of the nuclear power industry but to its own reputation.

100 Years Ago



ERNEST T. CHAPMAN

MR. E. T. CHAPMAN has met his death by an explosion in his laboratory at Rübeland in the Hartz. A letter containing this sad news has the following particulars:—

"As you are probably aware, Mr. Chapman's work was always in the laboratory, and it was there on the 25th inst. (June) that the accident occurred. On the morning of that day Mr. Chapman had a conversation with the gentlemen here, and shortly after this, at 11 A.M., the bomb-proof building in which he was with three workmen exploded with a tremendous crash. Mr. Chapman may perhaps have informed you that latterly he has been chiefly engaged with the production of nitric methyl-ether, with which he has been making various experiments, and we cannot explain the catastrophe otherwise than that he was not thoroughly aware of the great explosibility of this substance. The workmen present having also all perished, it is unfortunately not possible to obtain any details. The force of the explosion was so tremendous that all the surrounding buildings have been more or less injured, and about ten people seriously wounded."

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OLD WORLD

Moratorium on Whales in Doubt

THE twenty-fourth annual meeting of the International Whaling Commission opened in London this week with the prospect of a fierce battle over the proposal for a ten-year moratorium on whale hunting, with the United States urging a tough line against the hunters and with Japan and the Soviet Union muttering threats of disagreement, non-compliance and even walk-outs. The proposal for a moratorium was blessed by the Stockholm Conference on the Human Environment two weeks ago, when the Japanese delegation protested. The conference this week has been the first occasion on which the Russians have been able to say their piece.

It is therefore mildly encouraging that there has at last been some progress on the scheme for carrying international observers on whaling ships to monitor the quotas on which the IWC has already agreed. This scheme, first suggested by Norway nearly two decades ago, has already been implemented by South Africa, Australia, Iceland, Canada and Norway, and will be extended to ships of the Soviet Union and Japan in the Antarctic by the start of the whaling season which begins in December. In the past, for many years quotas fixed by the IWC were consistently exceeded.

There is also the admittedly remote possibility that the Soviet Union and Japan—the chief whaling nations—will agree to allow truly international observers aboard their ships, instead of simply swapping observers as at present; in view of their joint commercial interests, a more independent watch than at present would make the scheme more credible.

It was also confirmed at the opening of the conference on Monday that the Blue Whale Unit will be abandoned this year—a development welcomed by Mr Anthony Stodart, minister of state at the Ministry of Agriculture, Fisheries and Food, in his opening speech. Hitherto, quotas for whale hunters have been set in terms of the Blue Whale Unit, with the assumption that 1 blue whale=2 fin whales=2.5 humpback whales=6 sei whales. The result has been that whalers have been able to turn their attention to the smaller species as the larger species have been decimated. It remains to be seen whether this week's meeting will fix satisfactory quotas for the several species now being hunted, but it is at least known that separate quotas would

be acceptable to the Japanese. The commission is also considering regulating whale catches by the numbers in particular areas, as well as by species, although this is unlikely to be agreed this year.

These two sweeteners do not hide the sharp differences between the forty members of the commission on the moratorium. Britain promised to support the United States advocacy, although it is not yet known whether the British government will follow the Americans in prohibiting the importation of whale products, meat and corset stays included. But the Russians and Japanese between them now account for 80 per cent of all whales killed, and rest their case on the assertion that a moratorium is not necessary and that it would in any case be harmful to their

whaling industry. The lack of accurate information about whale stocks means that the conflict will not be resolved objectively.

This is why the sponsors of the proposal for a moratorium rely principally on the force of public opinion. Mr Russell Train, the chairman of the United States Council on Environmental Quality, began the proceedings this week with a proposal that the press should be admitted but he failed to find a seconder. Mr Train, who is new to the ways of diplomacy, will no doubt learn to prepare his ground more carefully. But the IWC did agree that observers and other representatives of environmental groups should speak before the conference got down to business, not at the end as has been the custom in the past. And the Friends of the Earth have been picketing the meeting, just in case delegates should forget that whales have friends.

The United States delegation began

METEOROLOGY

Whither the Weather?

LAST Monday was the warmest day in central London since October 23, 1971, with the temperature barely reaching 70° F, or 21° C. This year has been the first, since before the war, that the temperature in central London has not reached this mark by midsummer's day.

The chief reason for this cool period, which has daunted summer sportsmen in Britain, is that the Atlantic temperatures are some 2° C lower than average for this time of the year. Considering that the total variation in the June sea temperatures during the past 100 years has amounted to no more than 4.5° C, this is a considerable drop on the average, and it is no surprise to anyone, least of all the weather forecasters, that the British preoccupation with the weather has reached a new peak.

The lower Atlantic sea temperature affects the daily temperatures in Britain in two ways. First, and most obviously, the prevailing westerly winds blow into the western parts of Britain with an average temperature that is 2° C lower than usual for June. Second, depressions are more easily formed where the cold waters of the North Atlantic meet the warmer waters of the Gulf Stream—which this year are warmer than usual. The temperature difference actually speeds the

depressions along faster than usual, thus giving a lot of unsettled weather.

The root of the problem is the cold Atlantic waters. According to Mr Robert Ratcliffe of the Meteorological Office, this arises because of an unusually large amount of icebergs below 48° N latitude. At one stage in the spring the Canadians, who keep account and track icebergs for the benefit of shipping, reported that there were more than 600 icebergs in the North Atlantic below this latitude—an unprecedented number since 1912, when the Titanic sank. Many icebergs have now melted but it is unlikely that the temperature of the water will return to normal during this summer.

The large number of icebergs released into the Atlantic appears to have been a consequence of a depression over South Greenland for almost the whole of March and April, when the ice was breaking up. The accompanying winds drove the icebergs down the East coast of Labrador and into the Atlantic.

All is not lost, however, and the British summer may yet appear. The prospects for July, to be sure, are not good. Out of ten cold Junes, only four have been followed by warm Julys, four have been followed by average Julys and the remaining two have been cold.

Yet the summer may be saved if the winds for the next few weeks blow from the continent. British dependence on Europe is a meteorological reality.

the week without much hope for a moratorium. Although it thinks that the whaling nations are "nervous" of public opinion, it admits that there is a "lack of enthusiasm" for the moratorium. The biggest danger is that if the proposal is carried with the necessary two thirds majority, the Soviet Union and Japan may leave the IWC. So the best hope is that the conference will be able to tighten up on quotas and possibly agree on a moratorium on certain species.

NUCLEAR WEAPONS

France Presses On

IN spite of protests from all around the world and, even more uncharacteristically, from within France itself, the French government announced earlier this week that it will carry on with its planned atmospheric nuclear tests in the South Pacific. The French Defence Ministry announced on Sunday that the delay in beginning the tests since the warning to shipping to steer clear of the area was issued five days earlier was due solely to unfavourable weather. Thus were dashed the speculations at the weekend that France would back down, as it did last year, when that series of tests ended prematurely after protests from Peru.

The renewed threat by Peru to sever diplomatic relations with France will not cut much ice this time. Opinion in London is that France is now less worried about the possible loss of sales of military equipment to South America. In the past five years, France has sold Mirage fighter bombers to five Central American and South American countries, Peru included.

Opposition to the tests rests chiefly on the inevitable spread of fallout from atmospheric tests. France tested some weapons underground in the Sahara as long ago as 1962, but the costs are now higher and the Sahara is no longer available.

What are the new tests likely to accomplish? First, the French have still to develop a usable fusion warhead for their weapons, the eighteen land-based missiles and the several submarine rockets fitted with either fission or "doped fusion" warheads. The problem that the French are out to solve is how to develop a trigger for a fusion weapon.

It is also likely that the new tests are meant to perfect the miniaturization of fission nuclear warheads for France's Pluton weapons — mobile tactical weapons with a range of up to 75 miles. It was originally planned to have these weapons in service in 1972 but this date has gradually slipped, partly because of lack of money but also because of technical difficulties. The yield of one of

these weapons will be of the order of 20 kilotons.

Some support for the French claim that their tests do not produce significant radioactivity is given in a recent report from the New Zealand Department of Health (*Fallout from Nuclear Weapons Tests Conducted by France in the South Pacific from June to August 1971*, NRL-F/47). The New Zealand authorities have been monitoring the French tests since 1966 and the present report is concerned with the short lived activity, in particular the levels of iodine-131 which has a half life of 8 days. This fission fragment is a good guide to the strength and frequency of the tests.

Iodine was monitored both in milk and in cattle thyroid during the tests. The conclusions are that during the period of the tests, the average level of iodine in milk was twice that during the rest of the year, but this is insignificant compared with the limits suggested by the International Commission on Radiological Protection. For iodine in milk, the upper limit set in New Zealand is 200 picocuries per litre of milk which is less than one third the level approved by the ICRP; but the highest value recorded during the 1971 series of tests was 23 picocuries per litre with the average value countrywide being only 2 picocuries per litre. The average level when no tests were being conducted was 1 picocurie.

As expected, however, the levels of radioactivity found in Samoa, Tonga and Fiji, which are nearer the site of the French tests, are higher. During 1971, the highest levels were recorded at Apia in Samoa, with 47 picocuries per litre of milk during the test period.

In view of the short half life of iodine-131 and the fact that the prevailing winds at the French test site are westerly, thus blowing most of the radioactivity away from New Zealand, a better measure of the long term effects of the test is the strontium-90 and caesium-137 levels. The latest figures available from the New Zealand Department of Health give an average strontium-90 concentration for the twelve months ending in March 1971 of 6.5 picocuries of strontium per g of calcium in milk and a level of caesium-137 of 24 picocuries per g of potassium in milk. These figures are, respectively, 2.5 per cent and 0.3 per cent of the reference concentration levels.

EUROPEAN SCIENCE

A Common R & D Policy

THE European Commission has now produced concrete proposals for a European research and development

policy (see *Nature*, **237**, 202 and 362; 1972). The hope is that the coming enlargement of the European community will make it possible to improve on the sorry history of European co-operation in technology.

The new proposals, now to be considered by the Council of Ministers, provide for

- a European Research and Development Committee (ERDC), with some 20 members, to advise on policy;
- a European Science Foundation, to be run by the heads of the leading scientific institutions in the member countries, but with its own resources to support existing international establishments and to encourage centres of excellence such as Cern;
- regular meetings of ministers responsible for R and D to reach specific decisions and to advise on priorities, with a committee of senior government officials and commission representatives to review national programmes for research and development so as to align national policies more closely.

At the official level in the commission, there is great enthusiasm for co-operation. Mr Christopher Layton, Chef de Cabinet to Mr Altiero Spinelli, the commissioner responsible for industrial, scientific and technological affairs, is known to believe that work should begin on transport, computers, electronics and telecommunications. In short, the commission is hoping that the impending enlargement of the European Community will make it possible to compete effectively with United States companies such as IBM and ITT. How will this be done?

The concept of super-European companies is by no means new. The COST group consisting of the EEC countries and 13 other European nations considered the idea of a European computer some years ago, but rejected it on the grounds that the market for such a machine could not be predicted accurately enough, and because development costs were thought to be in the order of \$500 million a year over a period of about ten years. The essential novelty in the EEC's new approach is that member Governments would in future shoulder the burden of promoting coordination.

The new schemes will cost about \$110 million a year but not all of this will be new money. The Joint Research Centre at Ispra will still consume 40 per cent of the total, even though the staff is to be reduced to 1,600 and the three reactors shut down. The European Science Foundation is reckoned to cost only 0.5 per cent of the total budget.

APPOINTMENT

Astronomer Royal

SIR MARTIN RYLE, professor of radio astronomy at the University of Cambridge, is to be the next Astronomer Royal. The announcement made earlier this week will still speculation among the astronomy community in Britain about the post.

A year ago it was announced that following Sir Richard Woolley's resignation, the posts of Astronomer Royal and Director of the Royal Greenwich Observatory would be considered as separate appointments. In October 1971, Professor Margaret Burbidge of the University of Southern California was appointed as Director of the RGO and now Sir Martin's appointment completes the picture.

The post of Astronomer Royal carries no emolument and is of no fixed duration. The Astronomer Royal does not by right belong to any SRC committee, although Sir Martin is already a member of the Astronomy, Space and Radio Board of the Council and is chairman of the Royal Greenwich Observatory committee.

Sir Martin will be the twelfth Astronomer Royal since John Flamsteed, the first holder of the post, was appointed in 1675. He held office until 1719 to be succeeded by Edmund Halley (1719–1742), James Bradley (1742–1762), Nathaniel Bliss (1762–1765), Nevil Maskeline (1765–1811), John Pond (1811–1835), Sir George Airy (1835–1881), Sir William Christie (1881–1910), Sir Frank Dyson (1910–1933), Sir Harold Spencer Jones (1933–1955) and Sir Richard Woolley (1956–1971).

SELECT COMMITTEE

Harwell Defended

THE Select Committee on Science and Technology last week rejected the CBI's criticisms of the Atomic Energy Authority's establishment at Harwell. The CBI, in evidence before the select committee, had said that the closure of Harwell "would make no difference whatever to industry", but the Select Committee takes the side of the Institution of Professional Civil Servants, which considers that Harwell has a useful role to play in providing research and development resources supplementary to the needs of industry.

This support for Harwell is contained in the second of the expected four reports from the select committee following its long and thorough inquiry into the management of research and development in Britain (*The Non-Reactor Research and Development Activities of the Atomic Energy Authority*, HMSO, £0.15). The first report dealt with the

research councils, was published recently and the other one on the industrial establishments of the Department of Trade and Industry and one based on the evidence given to the committee by Mr John Davies, the Secretary of State for Trade and Industry, are promised within the next two weeks.

In spite of the affirmation of support for the research establishments of the Atomic Energy Authority, the select committee feels that more can and should be done to increase the proportion of work which is done at these establishments on a full repayment basis. During 1971–72, £15 million of the authority's total expenditure of £67.1 million was expected to be recouped from repayments. The predictions for 1976–77, however, are that the gross expenditure will drop to £53.4 million but the amount of contracted work will increase to £25.2 million. The select committee feels, however, that this rate of increase of contracted work should be speeded up and that the authority's work should be even more closely related to the needs of industry.

The select committee sees no difficulty in increasing contractual work from the government departments but it confesses that producing a commensurate increase from industry may not be so easy. To rectify this, the select committee recommends that the authority sets up an Industrial Advisory Committee to keep matters under review.

Even though most of the Atomic Energy Authority's work is already based on the customer-contractor principle, the select committee expressed some concern about the authority's research projects classified as "development of commercial agreements". The committee suggests that these projects be "scrutinized carefully" and that the Industrial Advisory Committee examine all projects to ensure "their relevance to potential industrial needs".

Of the authority's total annual expenditure of £52 million, only £12 million is spent on the non-reactor work considered in this report. This work, which falls into two categories, applied nuclear work and non-nuclear research, is considered by the committee to be so intimately connected to the reactor work of the authority, which accounts for the remaining £40 million, that it has considered it imprudent to make any sweeping recommendations about future developments of this work before the DTI takes action on the Vinter report. This report, which has now been in the hands of ministers for several weeks and which reputedly provides information for ministers to make up their minds on the next generation of nuclear power reactors which Britain should construct, is to remain unpublished, but there seems to be no obvious reason why a decision has been delayed so long. This

delay, according to the select committee, is having a bad effect on recruitment and morale.

SOVIET SCIENCE

Russian Computers

from our Soviet Correspondent

AUTOMATIC data processing is one of the fields of research and development especially stressed in the directives of the current five year plan of the Soviet Union. By 1975, the first stage of the computerization process should be complete, with the introduction of data processing systems in almost all ministries and government departments and also in several factories.

This is planned to be the first stage of a wider scheme. Interviewed in *Pravda* (June 10, 1972), Academician V. M. Glushkov emphasized that these systems are projected as units in an ultimate Union-wide data processing and control system, which would become virtually a model of the whole economic and productive structure of the Union.

Preparations for this integrated system are already going forward. Already factory and departmental computer systems are being designed and constructed with a view to their inclusion in the final state-wide network. In the Ukraine, which has been from the beginning one of the leading areas for Soviet computer research, a network of training schools for cyberneticians has recently been inaugurated.

POST OFFICE

Great Leap Forward?

by a Special Correspondent

Martlesham, Suffolk

THE British Post Office, for long the butt of those who complain that telephone subscribers have to wait too long to be connected to the network and then find that too large a proportion of their dialled calls go astray, and still struggling to reconcile its faith in digital transmission with the old fashioned Strowger switchgear with which most telephone exchanges are still equipped, seems nevertheless to be convinced that in the 1980s it will reach the promised land. The new laboratory, to which 550 people have already been moved from Dollis Hill, once a London suburb, is agog with plans for reconstructing the trunk transmission system. Mr John Bray, the director of research at the Post Office, talks like a man who might have echoed G. D. H. Cole's comment on his first visit to the Soviet Union—"I have seen the future and it works".

The move from Dollis Hill to Suffolk

has made it easier for the Post Office to take on new projects. For the time being, the research at Martlesham is housed in splendid buildings euphemistically described as temporary at the end of the runway from which Tizard, Lindemann and their colleagues put to the test during the First World War their schemes for pulling the early biplanes out of spins and stalls. The intention is that a further thousand of the Dollis Hill staff will move into the gigantic new laboratory now going up. Each of the seven floors of the main laboratory block will have more than 60,000 sq. ft of air conditioned working space with a maximum length of 550 ft. The scale of the building belies the impression that it will look like a cross between an airport and an airport hotel. The contractors have taken the intelligent precaution of providing on the site a building in which components and materials can be tested before use. Such anxieties as there are concern the problems of moving a research staff which, with families, will amount to more than two per cent of the present population of Suffolk into an area in which the schools are said by the immigrants, not necessarily correctly, to be less good than those in London, and where house prices have shared in the inflation of the past few months. One byproduct of the move, to be completed in 1974, is that this part of Suffolk will have a better telephone service than it can have dreamed of.

The schemes for rebuilding the trunk telephone network in Britain are known collectively and a little conspiratorially as "System X". The most glamorous component of the new programme is a

scheme for microwave transmission through hollow cylindrical waveguides and the new research laboratory is justly proud of its neat waveguide design—a helical winding of copper within a glass fibre case 5 cm in diameter. With luck, manufacturing costs will work out at £5 per metre. The plan is to lay 15 km of waveguide within a steel pipe conduit that will eventually connect Martlesham with the rest of the trunk network.

The immediate task is the design of repeater units. Eventually it may be possible to operate at all frequencies between 32 GHz and 110 GHz, but the first step is to test the system between 32 and 49 GHz. This should be enough to be going on with. The experimental section of the waveguide loop will be used to test alternative repeater units developed by the Post Office itself and by industrial companies. The trick is somehow to devise a way in which different sections of the frequency band can be selected by clever geometrical design and then amplified for onward transmission. Mr Bray says that the Post Office will be as objective as it can in assessing the merits of alternative designs and will, if necessary, be prepared to let its own ideas take second place to industry's.

It remains to be seen how quickly the demand on the trunk network in Britain will grow, but one virtue of the waveguide system is that repeaters can be added or made more complicated as the carrying capacity of the system has to be extended. In short, the capital cost of waveguide transmission systems does not all have to be spent at once, and the initial cost of the waveguide itself may be only a small part of the total.

For the more immediate future, the laboratory here is also working on schemes for the use of microwave transmission at 14, 17 and 20 GHz using atmospheric pathways. The intention is that there should be small microwave repeaters housed in containers much like dustbins mounted on masts 25 metres high. Repeaters will be separated by 5 or 10 km to make a network. The practical problem is to know how such a system will be affected by rainfall, and Martlesham is proud of what it describes as the most thorough investigation in the world to test the effects of rainfall on microwave transmission at these frequencies. South of the laboratory, there is being installed an array of rain gauges with associated transmission networks occupying a 12 km square. Along one arm of the network, there are rain gauges every 400 metres. The outcome should be not merely a set of design parameters for a transmission network but a more detailed knowledge of the microclimatology of Suffolk than could otherwise have been provided.

The Post Office's commitment to digital transmission is by now absolute, but everybody recognizes that it will be some time before digital techniques will live comfortably alongside some of the equipment still in service and likely to survive until the early years of the next century. In the long run, however, there should be immense economies to be won from the simplicity of switching techniques which digital transmission permits. It is much easier to select a desired signal from a data stream by counting pulses than by filtering out some specified band of frequencies. Stored program control is therefore bound to be another component of System X.

Precisely when will System X begin to take shape? From the plans already laid, it is clear that an accurate assessment of the several components of System X will not be possible until 1975 or thereabouts, when the waveguide trials are under way, when more is known of the effects of rain on transmission through the atmosphere and when there is a more confident feeling for switching techniques. At a guess, something like a quarter of the research and development budget at the Post Office is being spent on research and development in trunk transmission, the best part of £4 million a year. It would be entirely unreasonable to complain that the Post Office does not at this stage know precisely what it will be building in the 1980s. A few years from now, however, when the preliminary development is complete, the Post Office can expect that those who watch its activities closely will be anxious to ensure that enough is spent on the development of the new system.



Martlesham Heath—model of the new Post Office Research Station.

NEW WORLD

Technology and the New Federalism

by our Washington Correspondent

THE Nixon Administration's much publicized plans for bringing science and technology to bear on pressing domestic problems may lack an important link. Two recent reports and a conference have all concluded that state and local governments lack the expertise and the machinery for handling decisions involving science and technology. Federal policy on science seems to be formulated with scarcely any input from those ultimately responsible for carrying it out.

Those were the two chief complaints that ran through a conference of representatives from federal, state and local government, industry and academe held amid the rain and floods of Harrisburg last week. The rapidly rising waters of the Susquehanna River effectively cut off most exits from Harrisburg and held the conference together to the bitter end, thereby providing not only a topic of conversation but a captive audience.

State and local governments have partly sought and partly had thrust on them a growing stake in the application of science and technology. Indeed they are the final and perhaps the most important links in the federal government's new policies for applied science and technology. So much was acknowledged by President Nixon in his message on science and technology to Congress in March, when he said "we must appreciate that the progress we seek requires a new partnership in science and technology—one which brings together the federal government, private enterprise, state and local governments and our universities and research centres in a coordinated, cooperative effort to serve the national interest."

One reason why the states and localities have such a prominent part to play in the Administration's goal of binding science and technology to the national interest is that, unlike the efforts to develop military and aerospace capabilities, for example, the new objective is to tackle problems which are basically the responsibility of state and local governments. Another is that an important plank in the Nixon Administration's overall domestic policies is to forge new links between various levels of government, chiefly by giving the states and localities more discretion over the way in which their own problems are tackled. The revenue sharing plan, passed last week by the House of Representatives, would distribute some \$29,600 million in federal tax money to state and local govern-

ments, to spend as they wish and is the cornerstone of the policy. "New Federalism" is the phrase.

How do the states and localities measure up to their new role? According to a recent report by a committee of the Federal Council for Science and Technology, "state and local governments, to a large extent, are not prepared to handle this public technology role, and . . . the federal government is doing far too little to involve those units of government in the formulation of science and technology priorities addressed to domestic needs" (*Public Technology*, available from the US government Printing Office, 40¢). Similarly, an unusually candid report prepared by a committee under the chairmanship of William D. Carey of Arthur D. Little Inc. and published by the Council of State Governments, concluded that "state governments, in general, have demonstrated only a lukewarm interest in science and technology. Nowhere are these tools a key element in decision-making." (*Power to the States*, available from the Council of State Governments, Iron Works Pike, Lexington, Ky 40505, \$2.00.)

There are, however, signs of change. Indeed, this conference is one hopeful sign. But it became abundantly clear last week that there are many political and organizational obstacles facing state and local government officials who wish to experiment with technological innovations in the public sector or to stimulate investment in research and development in the private sector. One problem is the lack of scientific advice in the deliberations of either the state administrations or the state legislatures.

To be sure, nearly every state now boasts a science adviser to the governor, and 19 state governments even have a Governor's Science Advisory Committee. But most of the participants in the conference agreed with the statement in the report of the Council of State Governments that "unhappily, the evidence is that these advisers often have low visibility, are not involved in the budget process, operate distantly from the governor, and rarely associate with the legislature. Typically, they wait to be called upon and rarely take the initiative themselves." And the conference participants had to look no further than Pennsylvania for proof.

Pennsylvania is generally agreed to have one of the most sophisticated and effective science policy organizations in the United States. It has a forceful and able science adviser, Thomas G. Fox, Professor of Chemistry at Car-

negie-Mellon University, a Governor's Science Advisory Committee and a board called the Pennsylvania Science and Engineering Foundation (PSEF) which provides seed money to initiate research, forge links between government, industry and the universities and support programmes designed to solve specific domestic problems. PSEF was started four years ago with a budget of \$1.4 million, but in 1971, financial stringencies forced the Pennsylvania administration to cut its budget by half and the foundation has received only \$700,000 for the past two years. The budget request for 1973 again asked for \$1.4 million, but the state legislature has been very heavy with the budgetary axe in an effort to head off a tax increase and slashed PSEF's budget to £200,000—a pitiful amount and a telling index of the regard for science and technology in the state legislature. The Pennsylvania administration is, however, hopeful that at least some of the money will be reinstated.

On the other side of the fence, several participants in the conference, notably Jack M. Campbell, former Governor of New Mexico, Arthur J. Holland, Mayor of Trenton, New Jersey, and Victor Yarnell, formerly Mayor of Reading, Pa, pointed out the political barriers that face any state administrator who wishes to bring a technological innovation into the public sector. First, the innovation must pay dividends in the short term if it is to be politically appealing—a delay in producing dividends would not help reelection. Second, the innovation must have an excellent chance of success—failures in public policy are more conspicuous than successes. And, third, nearly every technological innovation—such as the computerization of a department of the state government—must be preceded by extensive preparation and education of the personnel involved. In short, the political risks that accompany innovation in the public sector are usually very great, and certainly sufficient to ensure that most city and state officials will err on the side of conservatism.

There is also the hoary problem of how the state universities, industry and government can be brought into partnership to help technology transfer and innovation. The problem has received more than its fair share of analysis and recommendations, but little progress has been made. The report of the Council of State Governments acknowledges that "the record of state-university relations is spotty, and memories are long on both sides of the relationship", but like the FCST report, it recommends

that a useful approach is to create public technology centres such as the Research Triangle in North Carolina. A federal programme which may be helpful in this area is the National Science Foundation's Technology Incentives Programme, but to judge by the awareness of the objectives and methods of the programme expressed at the conference, not too many people in the field consider it an answer.

What then, is to be done to help the states apply science and technology to solve their problems? The conference was, after all, billed as a national action conference. A resolutions committee, which met throughout the conference, is now chewing over the recommendations contained in the reports of the Council of State Governments and of the Federal Council for Science and Technology, together with other recommendations which were suggested at the conference, and it should have something useful to say under six headings. The committee will recommend state and local representation on federal policy councils and boards working in the science policy field, joint state and federal funding for science and technology projects in the states, new institutional arrangements between universities and state governments, utilization of federal laboratories for projects designed by state and local governments and federal support to state legislatures to help them appraise the implications of legislation involving science and technology.

Of these recommendations, perhaps the most important, both symbolically and operationally, is the suggestion that state and local governments should be in on the planning of federal programmes and policies. The complaint that the federal government decides on policies without taking sufficient account of user needs was constantly raised at the conference, and is brought into sharp focus by the fact that federal science policy decisions are having more and more effect on the states. Policies for the environment, health care delivery and transportation, for example, must all be carried out at the state and local level. In the past, however, the chief concern of science policy has been geared towards defence and space needs, and the states' role has been chiefly reduced to bidding for contracts. The report of the Council of State Governments acknowledges, nevertheless, that "within the past year . . . the President's Science Adviser has made unprecedented efforts to involve state governments in discussions of national policies.

The conference resolution would, however, substitute for the present limited and informal consultation, a network of user groups in mission-oriented

agencies which would help to plan the transfer of technology from the federal government to the states and localities. The state and local governments would also be able to have a role in the planning of public technology projects through a task force established by the National Science Foundation, if the recommendation of the Council of State Governments is accepted. The idea would be to set up a task force to plan some public technology pilot programmes in a few selected states, and to start the first projects going in the next fiscal year. Such programmes would be pilot projects to test the feasibility of suggestions such as those mulled over during Mr Magruder's planning of the New Technology Opportunities Programme.

One result of the two reports and of the conference is likely to be the designation of a lead agency to oversee the relationships between the federal government and the states, and to hammer out the basis for the new federalism in science. President Nixon has asked the Office of Science and Technology to lay the groundwork and the consensus at the conference was that the National Science Foundation should now assume the chief role.

CANCER RESEARCH

Detrick Unveiled

by our Washington Correspondent

SINCE President Nixon announced in October last year that the former biological warfare research centre at Fort Detrick, Maryland, would be converted for research on cancer, speculation has been rife about what would actually be done there. Last week, however, the National Cancer Institute announced that Litton Bionetics Inc, a subsidiary of Litton Industries, has been given the contract to operate the establishment for the National Cancer Institute, and Dr Frank J. Rauscher, jun., the NCI's new director, provided a few details of the research now planned.

The contract to Bionetics amounts to about \$7 million for the first year of operation—which starts immediately—and there will be jobs for 150 people by the end of July, 300 by the end of the year and as many as 600 in five years. Although the facility will be operated under contract to the National Cancer Institute, it will, to a great extent, be under the NCI's everyday control. The operation is like the arrangement by which the Atomic Energy Commission's Oak Ridge Laboratory is managed by Union Carbide, and it provides a convenient way of increasing the National Cancer Institute's staff complement without breaking the Administration's general rules for reducing the number of

people on the federal government's payroll.

Among the functions to be carried out at Fort Detrick are the large scale production of tumour viruses, including candidate viruses isolated from human tissue cultures. Since Fort Detrick was used for research on dangerous viruses, it contains excellent facilities for virus production and for keeping them isolated from the environment, and it is hoped that the facility for large scale virus production will allow tumour viruses to be made available to cancer workers on request. Similarly, the facility's extensive animal holding and production facilities will allow about 5,000 mice and 5,000 rats, hamsters and guinea pigs to be maintained for studies at NIH for periods up to three years. Another production function at Fort Detrick will be the preparation and analysis of chemical carcinogens for use in biochemical and immunological studies.

At a later stage, Fort Detrick will be used for straightforward cancer research. There will be an advanced systems laboratory for cancer researchers from the US and abroad who are invited to the National Cancer Institute to work on a wide range of research, including immunology, virology and chemotherapy. It is also hoped to develop chemotherapy research at Fort Detrick separately from the advanced systems laboratory. Another project will be aimed at the development of an efficient tissue culture method for determining the carcinogenicity of various substances.

Between November 1969, when President Nixon announced that the United States would no longer engage in research, production or stockpiling of biological weapons, and October last year, when he announced that Fort Detrick would be converted for cancer research, the future of the facility hung in the balance. Consequently, nearly all the staff who were employed there by the army for weapons research have left the area, and earlier this year the General Accounting Office published a report critical of the way in which the transfer of Fort Detrick from the army to the National Cancer Institute was conducted. The GAO report pointed out that in February, fewer than 600 of the original staff complement were still working at the research facility, in spite of the fact that many people had suggested that it would be desirable to keep the research teams together. The GAO suggested that one of the reasons for the delay in transferring and converting the facilities was that no agency wanted the responsibility of managing the facility—a problem that has been partially circumvented by turning it over to a contractor.

NEWS AND VIEWS

Links of Iron

PERUTZ's haemoglobin crystallography turns out to be mightily powerful juju, for not only has he now established the precise relation between the coordination state of the haem iron, the structural changes within the subunits, their disposition relative to each other, and the affinity of the molecule for its ligands, but he has also found answers to questions that have exercised inorganic chemists and spectroscopists since the days of the biochemical ice age. Two articles, one by Perutz on page 495 of this issue of *Nature*, and one by Perutz, Pulsinelli and Ranney in this week's *Nature New Biology* (237, 259; 1972), tell the story.

Some important pointers to the truth emerged from the structural properties of an abnormal human haemoglobin, known as M Milwaukee. The designation haemoglobin M implies that, as it comes out of the patient, the pigment contains ferric haems, which are unable to bind oxygen. In general this is a consequence of the substitution of tyrosine for histidine as a ligand for the iron. In the Milwaukee variant, however, the mutation, which occurs in the β chains, consists of the replacement of a valine by glutamic acid in the haem pocket, on the oxygen-binding side of the haem plane. From difference Fourier maps it can be deduced that the carboxylate group of the glutamic acid is a haem ligand, which interacts strongly with the ferric ion by virtue of its charge.

A more remarkable result, however, is that the crystal structure of deoxyhaemoglobin M Milwaukee is isomorphous with that of normal deoxyhaemoglobin, rather than oxy- or methaemoglobin, as one would expect on the basis of experience with other haemoglobin M variants, as well as artificial hybrids of deoxygenated α chains, and cyanomet- β chains. When the α chains are in the oxygenated state, however, the crystals become isomorphous with those of normal oxy- (or met-) haemoglobin. If now the deoxygenated form of the Milwaukee variant is in the characteristic deoxyhaemoglobin state of quaternary structure (in allosteric vernacular, the *T* state), one may surmise that the ferric subunits are in the corresponding conformational state (which Perutz designates *t*); but when the α chains are oxygenated, the sequence of events previously enumerated by Perutz—starting with the shift of the iron atom into the haem plane, which pulls its histidine ligand with it, and activates the system of rods, pulleys, cogs and levers that finally breaks the critical inter-chain salt links—is set in train. The consequence would be that the β chains would enter their other conformational state (*r*), and the tetramer as a whole its *R* structure.

The implication is that the iron atom in the ferric subunits can be made to move into or out of the haem plane according to the demands of the two conformations. This displacement should in turn be manifested in changes of the spin state of the iron, which is governed by the haem geometry, and consequently, if theoretical divinations are correct, of certain features in the visible absorption spectrum. In particular, bands at 630 and 1,000 nm have been identified with the low-spin condition, and when for example methaemoglobin, which in the acid

form has water in the sixth coordination position, is titrated through its spectroscopic *pK* to the high-spin hydroxyl form, the band shifts and fades in intensity. In deoxyhaemoglobin M Milwaukee the ferric chains do not give rise to either of these low-spin absorption bands. When, however, the α chains are liganded with carbon monoxide, the characteristic bands—due to the ferric β chains—appear. Thus a primary event at the α chain haems generates a spectroscopic and structural change in the β chains.

Furthermore, in the deoxygenated haemoglobin, the ferric β chains, being in the *T* state, are able to bind the cofactors, diphosphoglycerate (DPG) and inositol hexaphosphate (IHP). In binding, these stabilize the *T* structure, so that they are able to inhibit the above-mentioned spectral change. It thus seems that the "allosteric constant" (*L*), which is simply the equilibrium constant for the *T*-*R* transition, has an anomalous value in the Milwaukee variant. Other properties, such as the presence of a Bohr effect, can then be readily rationalized.

Perutz descants on the same theme in considering haem-haem interactions in general. He has shown by examination of the absorption spectra of ferrous-ferric hybrids of normal haemoglobin in the spin-sensitive regions that here too the ferric β chain responds to the ligation state of the ferrous α chain, and that the *T*-*R* equilibrium can be controlled by addition or removal of the cofactors. There is thus a reciprocal reaction or, as Perutz has it, a seesaw, involving on the one hand the balance of the high spin and low spin states of the pigment (manifestations of the position of the iron relative to the haem plane), and on the other that of the *R* and *T* conformations. The same reasoning holds also for the wholly ferrous state: in the *T* geometry (that of deoxyhaemoglobin) the constraints of conformation impose a large out-of-plane displacement of the iron, which inhibits the oxygenation reaction, and is thus responsible for the low oxygen affinity of the deoxyhaemoglobin.

An interesting byproduct of all this is an explanation for the hitherto somewhat supererogatory observations on hyperfine shifts in the haemoglobin proton magnetic resonance (PMR) spectrum. The phenomena that are observed during oxygenation may now be seen in terms of the change in the spin state of the iron, which governs the magnitude of the contact-shifts. Close examination of the shift profiles shows that they represent a two-state process. Moreover, the observations on ferrous-ferric hybrids, in which oxygenation of α chains affects the magnitudes of chemical shifts in the resonances arising from the β chains, also now become comprehensible. Moreover, in this week's *Nature New Biology* (237, 263; 1972) Lindstrom, Ho and Pisciotta report that the oxygenation of the α chains in haemoglobin M Milwaukee leads, as one is now entitled to expect, to marked changes in the features of the hyperfine-shifted PMR spectrum assigned to the ferric β chains. The manner in which such diverse observations now fall into place is one of the most pleasing aspects of Perutz's new theory.—From our Molecular Biology Correspondent.

Using the Sun to find Home

THE ability of a pigeon to find its way home from any direction over unfamiliar territory implies that the bird can do more than simply maintain a constant compass direction. It implies that the bird in some way knows where it is in relation to its home loft. How this is achieved is still completely unknown.

One of the few theories to explain this ability is the Sun navigation hypothesis of G. V. T. Matthews (for example, *J. Exp. Biol.*, **28**, 508; 1951). An important part of this theory concerns how the birds know whether they have been transported to the north or to the south of their home. Matthews proposed that they did this by remembering the noon altitude of the Sun at their home loft and then comparing it with that of the Sun where they were released (if it happened not to be noon when they were released, the birds were held to observe the Sun for a short time and then to extrapolate its arc to obtain the noon position). If the noon position of the Sun were lower in the sky than it had been at their home, the birds would know that they had been moved northwards; if it were higher, they had been displaced southwards.

Recent experiments have cast doubt on the ability of birds to obtain information about latitude displacements in this way. Walcott and Michener, for example (*J. Exp. Biol.*, **54**, 291; 1971), attempted to delude pigeons about the noon altitude of their home Sun by allowing them to see the Sun only after it had been deflected off a mirror so that it was too low or too high in the sky. If the birds were behaving in the way that Matthews suggested, they should then have had a false picture of what the home Sun looked like and should therefore have miscalculated how far north or south of home they were when moved to a strange place. The navigational abilities of these pigeons were not affected, however (possibly because they had remembered the true arc of the home Sun before the mirror manipulations began).

In a recent issue of *Nature* (**237**, 405; 1972) A. Whiten, of the University of Bristol, provides convincing evidence that pigeons can make

use of the Sun to give them information about their latitude. Pigeons were confined in a box and rewarded for pecking at a key when pointing towards home but not when pointing away from it. Box and pigeons were transported to three widely separated sites and it was found that they could indeed tell which direction they were facing. They could easily discriminate between "away from home" and "towards home", even though no landmarks were visible and other directional cues, apart from the Sun, were excluded. When the apparent position of this Sun was altered with a periscope, the birds believed this false Sun and pecked when, according to this reflected Sun, they were pointing homewards. When they could not see the Sun at all, the discrimination failed completely. They showed the same ability in the laboratory with an artificial sun moving across a plastic dome.

The question of how birds know whether they have been displaced to the north or to the south is still open. But this new work at least shows that birds can use the Sun to navigate.—From our Animal Behaviour Correspondent.

PROSTAGLANDINS

Promise but no Miracles

from a Correspondent

EXCITEMENT about the potential of prostaglandins as wonder drugs for abortion and contraception should subside to a more modest hope for favourable future developments in the wake of a symposium held on June 12–14. During these three days the results of two years of clinical trials were exposed to the critical view of an international gathering of people who have been assessing the efficacy of these drugs in human reproduction.

The sixty scientists—only six of them women in spite of the obvious female bias of the work reported—were guests of the Upjohn Company in the luxurious surroundings of Brook Lodge in Augusta, Michigan. The company's interest in prostaglandins began fifteen years ago, and is manifest in the acknowledgments of grateful researchers whose publications conclude with thanks to Dr J. E. Pike of the Up-

john Co. for the generous supply of prostaglandins.

The profusion of such acknowledgments is evidence of the enterprise of Upjohn in setting up a research programme when prostaglandins were unglamorous substances known to cause muscle to contract. The Upjohn team had worked out two methods for synthesizing prostaglandins by 1966, one involving the use of seminal vesicles, the other being a total synthesis method. This large group of 20-carbon carboxylic fatty acids was discovered in 1930 by two New York gynaecologists, Raphael Kurzrok and Charles C. Lieb, and named by Ulf von Euler of the Karolinska Institute in Stockholm. At the Brook Lodge symposium, which coincided with Upjohn's applications to market the first prostaglandins in several countries, one of von Euler's colleagues, Sune Bergström, was a guest of honour.

The first day was packed with reports from groups that have been using prostaglandins E_2 and $F_{2\alpha}$ to induce labour in women whose pregnancies have reached term and who need, for some reason, to be delivered immediately. The pituitary hormone oxytocin is used intravenously as a routine means of inducing labour, and results so far with prostaglandins do not indicate any definite advantage by this route of administration. Given orally, however, prostaglandins seem to have a potential advantage.

In a double blind comparison involving 169 women, reported by Dr G. G. Anderson (Yale University School of Medicine), prostaglandins E_2 and $F_{2\alpha}$ administered intravenously were equally effective inducers of labour as oxytocin. Anderson and his colleagues found that the prostaglandins seemed to be as safe as oxytocin, and reported some innocuous hypertonus as the only side effect. Dr A. I. Sherman (Sinai Hospital, Detroit) reported similar results from a double blind study. His group found that prostaglandin $F_{2\alpha}$ compared very favourably with oxytocin for the induction of labour at term.

Dr W. N. Spellacy (University of Miami) sounded a note of warning when reporting his own double blind intravenous study with oxytocin and prostaglandin $F_{2\alpha}$ used to induce labour in 222 women. With prostaglandin he found a greater proportion of maternal complications in the form of uterine hypertonus, hot flushes and infusion vein phlebitis. There was also a greater need to perform Caesarian sections in these women. Spellacy recommended

the obstetrical community to exercise caution in using prostaglandins to induce labour.

Successful induction of labour in thirty women by prostaglandins E_2 and $F_{2\alpha}$ given intravenously was also reported by Dr J. A. Elias (King's College Hospital Medical School, London). The only troublesome side effect was phlebitis at the site of infusion in eight cases. Elias also reported his favourable experience with prostaglandins given orally. The trials proved very effective, with no observable adverse effects.

The greatest success with both the intravenous and oral routes of administration was reported by one of the pioneers in the use of prostaglandins, Dr S. M. M. Karim (Makerere University College Medical School, Kampala). These drugs have been used routinely in his hospital for the past four years. For two years his obstetrical colleagues used intravenous infusion, but now oral prostaglandins are in routine use for the induction of labour. Karim's team uses smaller doses than were reported by other participants and for this reason he was not surprised by their less favourable findings.

Varying success was also reported for the induction of abortions, although in this case prostaglandins seem to offer a valuable alternative to existing methods, particularly during the second three months of pregnancy. The most satisfactory methods of administration have proved to be by a catheter passed through the abdomen into the uterus, either intra- or extra-amniotically. Groups at the Radcliffe Infirmary, Oxford, and the Karolinska Hospital, Stockholm, led by Drs M. P. Embrey and M. Bygdeman respectively, reported successful abortions in approximately 90 per cent of cases within 36 hours using the extra-amniotic route. This method, however, involves a significant incidence of side effects in the form of nausea, vomiting and diarrhoea.

Intra-amniotic administration of prostaglandins, however, seems equally effective during the second trimester, and produces a more acceptable incidence of side effects. Anderson reported excellent results with eighty patients. Dr F. Naftolin (University of California, San Diego) was also optimistic about this method and Karim reported considerable success in Kampala. Dr C. H. Hendricks (University of North Carolina) thought it not unreasonable to hope that within the next few years the intra-uterine application of a natural prostaglandin, or a prostaglandin analogue, may become, in many parts of the world, the preferred method for inducing abortion during the second three months of pregnancy.

Intravaginal administration of prostaglandins has proved disappointing,

chiefly because of the high incidence of side effects. Dr R. J. Pion (University of Hawaii), who tested the instillation of prostaglandin $F_{2\alpha}$ into the vagina, and Dr N. C. Wiquist (Karolinska Hospital, Stockholm), who reported the use of suppositories containing the same prostaglandin, both had very modest success. This method, however, will continue to attract research attention, as will oral administration, because if prostaglandins are ever to provide a means by which women can terminate their own pregnancies in the first few weeks, one of these methods will have to be used.

At present prostaglandins cannot compete with vacuum aspiration as the most effective means of abortion during very early pregnancy. The day when a prostaglandin pill or suppository can be taken as a routine means of regulating menstruation (the much vaunted "morning-after pill") is clearly a very distant prospect. Among people who have been looking for a simple, safe and acceptable means of using prostaglandins as an abortifacient cum contraceptive, Bygdeman and Karim agreed that such a goal had not yet been reached.

With this aim in view, however, researchers are now looking towards the analogues of prostaglandins E_2 and $F_{2\alpha}$ that Upjohn is developing.

QUANTUM MECHANICS

Dirac 40 Years On

from a Correspondent

IN mathematical physics, genius usually belongs to youth. Newton resigned the Lucasian chair and took to theology and the Royal Mint while still middle aged; Maxwell produced his thermodynamic relations while still an undergraduate; Einstein discovered special relativity and the photoelectric effect while still in his twenties and mispent his age on a unified field theory which did not work.

Dirac is therefore exceptional in writing two articles of the highest importance shortly after retiring from the Lucasian Professorship in Cambridge. He now describes (*Proc. Roy. Soc. A.*, **322**, 435; 1971; and **328**, 1; 1972) a quantum mechanical model of a class of fundamental particles whose mass is necessarily positive and which therefore have no corresponding antiparticles. Antiparticles and the possibility of negative mass or energy were discovered by Dirac more than forty years ago and his results were confirmed experimentally shortly afterwards. He won the Nobel prize for the discovery, and on that occasion a journalist asked him whether any of his work was explicable to the layman. After a little thought he answered "No", and with that gentle

Strand Switches in SV40 Transcription

IN a lysogenized *Escherichia coli* cell the DNA genome of a lysogenic phage is covalently integrated into the bacterial chromosome and the molecular events which lead to integration have been thoroughly characterized. In an animal cell transformed by SV40 or polyoma virus, the viral genome becomes stably associated with and probably integrated into the DNA of one or more host chromosomes. Although the molecular mechanisms which result in this integration remain obscure, tumour virologists have freely adapted models of lysogeny to explain events leading to transformation. No doubt after they have read what Khoury and Martin have to say in next Wednesday's *Nature New Biology* (July 5) their conviction that the molecular biology of the small DNA tumour viruses is essentially similar to that of the DNA phages will be even stronger.

Khoury and Martin have shown, by sophisticated hybridization techniques, first, that when SV40 virus replicates in permissive cells segments of both of the two strands of the double-helical DNA are transcribed into viral RNA and, second, that the total RNA made off both strands is complementary to 50 per cent of the total genome. By contrast, when superhelical SV40 DNA extracted

from virions is transcribed *in vitro* by DNA dependent RNA polymerase from *E. coli* only one of the two SV40 DNA strands is transcribed but its whole length is transcribed. In other words, during the lytic cycle the transcription of SV40 DNA involves strand switching which does not occur *in vitro* with a heterologous polymerase.

There are, of course, numerous precedents for such strand switching during the *in vivo* transcription of the genomes of coliphages such as T4 and lambda. These phage genomes are, however, many times larger than that of SV40. It is something of a surprise, therefore, that the transcription of a genome as small as that of SV40 (it can code only for about 200,000 daltons of polypeptide) should involve strand switching especially because the transcription of the replicative form DNA of phage ϕ X174, which is almost exactly the same size as SV40 DNA, involves only one DNA strand.

At least two other groups have apparently made observations similar to those of Khoury and Martin, and these groups are now all attempting to elucidate the mechanism and significance of transcriptional strand switching not only during the lytic cycle of SV40, but also in cells transformed by this virus.

monosyllable unwittingly provided a clue to the nature of his genius.

Dirac's ideas are invariably very simple, simpler indeed than usual pre-conceptions of distance, position, time and so on, and followed up with complete literalness and disregard of commonsense. He deduced the existence of antimatter by a very long argument which began with a deduction that the wave equation must be first order in the distance variables. At that point, where most physicists would have written "which is absurd", Dirac wrote "therefore".

Dirac's work of forty years ago showed that all particles of a certain degree of simplicity have no internal degrees of freedom other than a spin, which must be half integral provided that the position of the particles is, in a certain sense, observable: this is true of electrons.

Dirac, in his new articles, allows more internal degrees of freedom characteristic of two harmonic oscillators and proceeds to write down the simplest conceivable wave equation consistent with that assumption. This equation bears a strong formal resemblance to the Dirac equation for the electron. It is a tribute to the methods discovered forty years ago that exactly the same methods elucidate quite different properties deduced from the new hypothesis and show that the resemblance was entirely formal. The new particles have positive mass, integral spin, zero orbital angular momentum and spherical symmetry.

It is evident that the fertility of the mathematical methods discovered by Dirac when a young man is not yet exhausted. But, recalling his remarks to the journalist, further explanation will not be attempted here.

AGGRESSION

Keeping Up the Rivalry

from our Animal Behaviour Correspondent

THERE has been much controversy recently about the causation of aggression in animals. Konrad Lorenz, in his book *On Aggression*, claims that animals have an aggressive "drive" which builds up and is discharged through acts of aggression or their substitutes. His view of aggression is of a force within the animal, as inevitable and basic as hunger, and something which, if not allowed expression, will build up to dangerous levels. Other authors, such as the American J. P. Scott, see the aggression of an animal as being elicited by external stimuli and unlikely to occur unless these stimuli are provided.

W. Heiligenberg and U. Kramer (*J. Comp. Physiol.*, 77, 332; 1972) have performed some experiments which go strongly against Lorenz's views of a

spontaneous build up of aggression and illustrate for the cichlid fish *Haplochromis burtoni* the great importance of the right external stimuli in keeping the aggressive tendency high. They used the rate at which male fish would attack small test fish as a measure of how aggressive the males were. If the males were isolated from other adults for a few weeks, their aggressiveness fell to a very low level, which is, of course, the very opposite of what would be expected if their aggressiveness were, as Lorenz claims, spontaneously "building up". The external stimulus of another adult fish seems to be needed to keep aggression high.

Functionally, this behaviour would be favoured by natural selection. When a male holding a territory is approached by a rival, his readiness to attack will be increased and this will in turn raise the probability of his winning the encounter. The aggressiveness will thus be adjusted to the number of encounters with other males in which a male has to participate in order to keep the territory free of rivals.

Differential Recognition Immune Responses

It is common among the various groups which study tumour immunology to suppose that the putative response of a tumour-bearing organism to its tumour involves two components, humoral and cellular. It is further envisaged that the cellular component consists of cytotoxic lymphocytes of which the activity may be either impeded or potentiated by antibody. This hypothesis, comfortable in the sense that it can be used to fit all occasions, also usefully points to the necessity for measurement of both components of the immune process.

It is clear that, if the hypothesis bears any relation to the truth, it is desirable to interfere with the response to the tumour, not by some blanket type of immunosuppressive or immunopotentiating regimen but by effecting separate regulation of the two components of the response. For example, if antibodies are blocking what would otherwise be an effective cytotoxic cell population, the antibody response must be reduced without concomitant reduction of activity by the cytotoxic cells. In a somewhat indirect way the report by Thompson *et al.* in *Nature New Biology* next Wednesday (July 5) impinges on this problem.

The humoral immune responses of guinea-pigs to lysozyme and its reduced and S-carboxymethylated derivative (CM-lysozyme) consist of a non-cross-reactive array of antibody molecules. Thompson *et al.*, however, show that in other ways the immune response to lysozyme and the partly denatured CM-lysozyme is cross-reactive. Spleen cells

ACETYLCHOLINE

Dual Action Synapse

from our Neurochemistry Correspondent

THE use of comparatively simple preparations from the nervous system of invertebrates has many advantages for the study of basic mechanisms of synaptic transmission, chiefly because of the relative precision with which connexions can be described. The ganglia of *Aplysia* have proved an outstanding example, although from the richness of the responses of preparations to applied chemicals, they may have seemed to be something of a pharmacologist's Pandora's box. The use of such preparations, however, is vindicated in an article describing a novel type of chemical synapse discovered in the buccal ganglion of *Aplysia*, where the same transmitter causes concurrent excitation and inhibition (D. Gardner and E. R. Kandel, *Science*, 176, 675; 1972).

In the ganglia of *Aplysia* single neurones innervate a large population of follower cells. Kandel and his col-

from immunized animals responding *in vitro* to either the homologous or heterologous antigen, for example, give exactly the same response, as measured by incorporation of tritiated thymidine. Delayed skin reactions of differentially immunized animals were almost exactly alike, whichever of the two species of antigen was used to elicit the recall response.

The production of a factor which inhibits the migration of peritoneal exudate cells could be successfully elicited from sensitized cells whichever of the antigens was added to the reacting cells. Mice which had been rendered tolerant to lysozyme were found to be cross-tolerant to CM-lysozyme.

The inference from these findings is that although the antigenic recognition system which is involved in humoral antibody production failed to find any recognition sites in common between lysozyme and CM-lysozyme, nevertheless the recognition system(s) which was or were involved in the other tests did find common sites.

Thompson *et al.* point out that their findings indicate a fundamental difference in the mode of recognition of antigens which induce humoral and cellular immunity. They specifically suggest that this may be of prime importance in situations in which it is wished to augment a cellular response by use of a partly denatured immunogen, without affecting the humoral response which, on their evidence, will not recognize any determinants in common in the native and denatured material.

leagues have previously described interneurons in the buccal and abdominal ganglia which mediate excitatory actions at synapses with certain of the follower cells, and inhibitory actions at others. According to Dale's principle, a single neurone releases the same transmitter at all of its terminals, and this seems to hold true in the present case, because both excitatory and inhibitory synaptic potentials resulting from activation of the interneurons can be mimicked in the appropriate follower cells by the application of acetylcholine (ACh).

The differences in response must therefore be determined at the post-synaptic receptor sites, and have been shown by the authors to be associated with different changes in the ionic conductances of the postsynaptic membrane, similar to those observed for excitatory and inhibitory responses in many other systems. Thus, excitation results from a depolarizing post-synaptic potential (EPSP), with a reversal potential of around -14 mV for cells with a mean resting potential of -60 mV, which probably depends on an increase in membrane conductance to sodium ions because it is reduced or abolished in the presence of low-sodium media. Inhibition, however, is associated with a hyperpolarizing potential (IPSP), which reverses at about -74 mV, and is found to be blocked in the absence of external chloride ions, suggesting that an increase in chloride conductance is involved.

Gardner and Kandel identified two types of excitatory follower cell in the buccal ganglion of *Aplysia*; one showed a conventional single component EPSP, but in the other type the potential response proved on closer inspection to be diphasic, with a small late inhibitory component following the initial depolarization. This second component could be made more evident by reduction of the membrane potential, which caused it to increase in size. The reversal potential for the first component had a mean of -10 mV, and that for the second component was -63 mV. Both actions appeared to result from direct connexions, because they were unaffected by high calcium solutions which would be expected to reduce the response if it were dependent on conduction in intercalated interneurons.

Iontophoretic applications of ACh to those follower cells which showed a two-component synaptic potential also caused a diphasic depolarizing-hyperpolarizing response. Moreover, each of the two components could be selectively blocked by different cholinergic blocking agents; hexamethonium, which blocks the EPSP in purely excitatory follower cells but not the IPSP in purely inhibitory follower cells, also blocked the excitatory component in those cells

showing diphasic responses without affecting the inhibitory component. Conversely, curare selectively blocked the inhibitory component in dual follower cells. This suggests that the diphasic response in dual follower cells results from the simultaneous combination of ACh with the two types of post-synaptic cholinergic receptor which are at present singly in purely excitatory or inhibitory cells.

A similar "dual" synapse involving both excitatory and inhibitory cholinergic receptors has been previously described by Kandel and his colleagues in the abdominal ganglion of *Aplysia*, but in this case simultaneous activation of both types of receptor during pre-synaptic stimulation rarely occurs because of differences in their threshold and rate of desensitization. Thus, at low firing rates, pure EPSPs are obtained, and at higher rates pure IPSPs result, presumably because the excitatory receptors are desensitized. Thus the physiological effectiveness of the synapse is determined by the state of sensitization of the two types of receptor.

The two components of the dual synapse in the buccal ganglion also vary in their response to repeated pre-synaptic stimulation. Although both components are reduced the decrease is greater for the hyperpolarizing component, presumably again because of preferential desensitization at high firing rates, but in this case of the inhibitory receptors. Thus the sign of the

buccal ganglion dual synapse, like that of its counterpart in the abdominal ganglion, is frequency dependent, although in the reverse way. Furthermore, it is dependent on the membrane potential, tending to be primarily excitatory near the membrane potential but becoming progressively more inhibitory as the membrane is depolarized by other inputs.

As is pointed out by Gardner and Kandel, the moral of *Aplysia's* tale is that a wide variety of synaptic actions can be triggered by a single chemical transmitter and the response obtained is dependent on the types of postsynaptic receptor, the combination of receptors and their sequence of activation.

ENVIRONMENTAL HEALTH

Moderation is the Word

from a Correspondent

IF the tone of the recent sixth annual conference on trace substances in environmental health at the University of Missouri, June 13–15, is an indicator, the near hysteria that has gripped segments of the scientific community and public at large in the United States during the past several years seems to be gradually easing.

In discussing the geography of insecticides and storage of organochlorine compounds, Dr M. Wasserman (Hebrew University, Jerusalem) pointed out the tendency of biological systems to stabilize internally in spite of qualitative

Aggregation Factors and Receptors

IN *Nature New Biology* next Wednesday (July 5), Pessac and Defendi describe a set of experiments which strongly support the notion that cells aggregate because they have on their surfaces receptors which bind so-called aggregation factors. Moreover, these workers also suggest that the potential of malignant cells to form solid tumours may be directly related to an increase in the number of surface receptors which bind aggregation factors.

The observation that the medium in which cells have been cultivated promotes cell aggregation—presumably because cells release into their medium aggregation factors and thereby condition it—has been made repeatedly and in many cases aggregation factors or more precisely conditional media have been shown to act on heterologous as well as homologous cells. But how do these factors act? Pessac and Defendi, who favoured the hypothesis that they bind to receptor sites on the cell surface and cross-link one cell to the next, set about answering this question by investigating the properties of four lines of murine lymphoid cells which do not

aggregate spontaneously in media lacking serum.

Because medium conditioned by these lymphoid cells does not promote the aggregation of any cells they conclude that the cells of these four lines do not secrete aggregation factors. Furthermore, cells of three of the four lines do not aggregate when placed in medium known to contain aggregation factors. Cells of the fourth line do, however, aggregate under these conditions. Pessac and Defendi conclude therefore that when cells fail to aggregate they either fail to make aggregation factor or lack surface receptors or both, and that aggregation factors and receptors are distinct structurally.

The fact that receptors can be destroyed *in situ* at the cell surface by proteases whereas aggregation factors are not susceptible to proteolytic enzymes but are inactivated by hyaluronidase also indicates that receptors and factors are chemically distinct. Finally, Pessac and Defendi comment that it may be significant that highly malignant cells aggregate more readily than do weakly malignant cells.

changes in the external environment. Wherever DDT has been used, it is found stored in the adipose tissue of man and animals, yet there is little convincing evidence for detrimental effects. The fortuitous observation, that spraying animals with DDT shortens sleeping time induced by phenobarbital, initiated research that increased the understanding of how drug metabolizing enzymes of the liver influence beneficial as well as harmful substances entering the body, and pointed the way toward more effective use of therapeutic agents.

Several epidemiologists have used the results of field studies or autopsies in an attempt to link environmental factors to disease in man. Dr C. Heath, jun. (Center for Disease Control, Atlanta, Georgia), described forty-two cases of childhood leukaemia or lymphoma recorded during the past thirteen years in DeKalb County, Georgia, with some indication of clustering. Dr W. R. Cameron (Florida Department of Health) described the results of a study in elementary and high school students screened for hypertension in which hard water, particularly due to lithium, was associated with a beneficial effect. Dr A. W. Voors (University of North Carolina) suggested that increased ratios of cadmium to zinc in tissues from human autopsies could be related to the severity of cardiovascular disease. In most cases, however, epidemiology seemed to be on rather tenuous grounds and a need for caution in interpreting the results of studies was noted.

The current status of mercury and environmental pollution was discussed by Dr G. Lofroth (Stockholm, Sweden); he described the enormous variation in the half life of mercury in man and other species, including fish, and indicated that studies so far imply that man-made pollution of the ocean with mercury is very limited and that sewer sludge should be given considerably more attention.

Other presentations emphasized the potential for trace metal contamination from industrial facilities and mining operations and a need for continuing monitoring systems, but one was left with the impression that much of this is coming under control. The interaction of trace elements continues to receive high priority as being more significant than the concentration of single elements *per se*. Dr D. W. Klauder (University of Cincinnati) implicated lead in copper deficiency associated with swayback in sheep. The role of zinc in the healing process has been studied by many investigators; synthesis of collagen requires zinc, as Dr J. M. Hsu (Johns Hopkins University) reported, and this may be influenced by the pituitary-adrenal axis, according to Dr A. Flynn (Case Western Reserve University). A highly signifi-

cant report from Drs C. E. Hunt and J. M. Narvia (University of Alabama) detailed prenatal effects of strontium, molybdenum, lithium and boron on the incidence of dental caries in post-natal rats, and suggested strongly that dental caries may be influenced markedly by the interaction of trace elements during intrauterine development.

On health effects of trace substances, Dr E. A. Doisy (St Louis University) described the influence of manganese on biosynthesis of prothrombin and cholesterol in the biological system. The beneficial effects of trace elements, acting through enzyme systems, and detrimental aspects of heavy metals, especially cadmium and zinc, were described. Lead in illegal alcohol was associated with severe derangements of the endocrine system, said Dr H. Sandstead (Grand Forks, North Dakota), particularly the pituitary, adrenal and juxtaglomerular apparatus. The protective effects of selenium against heavy metal toxicities and the role of selenium in host defence mechanisms were discussed, as well as the lack of significant amounts of selenium in the edible portions of poultry fed growth promoting concentrations of selenium in the diet.

Analytical methodology was discussed in relation to its shortcomings and attempts to standardize results regardless of methods were urged for all laboratories. Finally, a need for conceptual thinking in epidemiological research was suggested and participants were urged to consider differences in biological availability of trace substances and metals in the human environment.

Oncolytic Viruses

THE idea that it may eventually be possible to kill certain tumour cells by infecting them with lytic viruses which for one reason or another more readily infect tumour cells than normal cells has often been raised over the past several years. And although little of therapeutic value has so far emerged the idea still has its champions, including Taylor and his colleagues at Indiana University who, in next Wednesday's edition of *Nature New Biology* (July 5) describe their latest investigations of the oncolytic action of bovine enterovirus on both human and murine tumour cells.

In 1970 and 1971 Taylor's group reported experiments which indicated that bovine enterovirus preferentially absorbs to murine tumour cells, Ehrlich carcinoma and sarcoma cells growing either as a solid tumour or an ascites tumour, and replicates in them and kills them. To see if this phenomenon is of more general significance they have now injected bovine enterovirus into in-

MESSANGER RNA

The Missing Poly A Tail

from our Cell Biology Correspondent

THESE days, when the magic words polyadenylic acid are whispered to the trendy biochemist, he immediately starts thinking, and more often than not talking, about messenger RNAs. It seems that many, perhaps indeed virtually all, mRNAs in animal cells have at their 3' termini a sequence of 150-200 adenylic acid residues which, it is believed, are added after transcription to the tails of heterogeneous nuclear RNA precursor molecules. And although nobody really knows why a poly A tail is required by a messenger, it is widely speculated that the poly A may play some part in the cleavage maturation and/or export of messenger molecules from the nucleus. Testing these plausible speculations will obviously not be an easy task, but as Adesnik and Darnell (*J. Mol. Biol.*, **67**, 397; 1972) point out a comparison of the metabolism of "9S" histone mRNA with that of other mRNAs ought to be particularly instructive.

Adesnik and Darnell isolated from HeLa cells so called "9S" histone messenger RNA, a collection of RNA molecules which, as others have shown, appears in small cytoplasmic polyribosomes during a cell's S-phase and which by virtue of its size, kinetics and other properties has been tentatively identified as the messenger for histones. This RNA comprises chains of about 400 nucleotides; if the molecules terminated in a tail of about 180

bred mice bearing various transplantable tumours and assayed subsequent tumour growth. They have also measured the response of primary cultures of various human and murine tumours and of cells of human and murine cell lines to infection with this virus.

Taylor and his colleagues find that although normal murine and human cells do not in general suffer any detectable cytopathic effect after exposure to bovine enterovirus, many human and murine tumour cells in culture are infected and lysed by the virus. Moreover, four bovine enterovirus caused the lysis of cells of four transplantable tumours growing in mice.

Sedmak, Taylor, Mealey and Chen are sufficiently encouraged by these results to conclude guardedly that there is still a possibility "that eventually viral oncolysis will play some role in human cancer therapy". Whether such optimism is really justified remains, of course, to be seen.

adenylic acid residues some 50 per cent of their nucleotides should be AMP residues, whereas in fact their base composition is 26.2 per cent A, 22.4 per cent C, 31.4 per cent G and 21 per cent U and total HeLa cell messenger RNA contains 30.1 per cent A. Moreover, digestion of this 9S RNA, labelled with ^3H -adenosine, by pancreatic ribonuclease followed by polyacrylamide gel electrophoresis, failed to reveal any polyadenylic acid and it seems unlikely that more than about eight of the hundred AMP residues in this RNA occur at adjacent sites.

Apart from this absence of poly A, Adesnik and Darnell draw attention to two other characteristics of putative histone messenger. First, its exit time from the nucleus is considerably shorter than that of other messenger RNAs, as befits a molecule which does not have to wait while poly A is added to its 3' terminus. Second, in spite of the lack of poly A and the short exit time, the histone mRNA seem to be derived from larger precursor molecules because they continue to accumulate in small cytoplasmic polysomes after all RNA synthesis has been blocked with actinomycin D.

It is not yet known whether the absence of poly A from histone messenger is a unique characteristic, but whatever the case this RNA provides, so to speak, a yardstick against which the metabolism of other poly A containing messengers can be compared and from such comparisons it may be possible to obtain some experimental evidence about the role of poly A in messengers.

All these arguments rest on the assumption that this 9S RNA is indeed a messenger, and no doubt Adesnik and Darnell would be among the first to admit that at present this claim rests entirely on circumstantial evidence. What is needed is a demonstration that 9S putative histone mRNA will programme the synthesis of histone proteins in a cell free system or in a heterologous cell system, and it can confidently be anticipated that strenuous efforts will be made to obtain unequivocal evidence for such activity.

The *Xenopus* oocyte system developed by Marbaix, Gurdon and their collaborators, in particular, seems to offer an opportunity of proving the messenger activity of this RNA. Not only is 9S rabbit reticulocyte RNA translated into both α and β rabbit globins, as Marbaix and Lane have now shown beyond all question (*J. Mol. Biol.*, **67**, 517; 1972), but also calf lens crystallin messenger RNA is translated in *Xenopus* oocytes. This has just been reported (*Proc. US Nat. Acad. Sci.*, **69**, 1606; 1972) by Berns, Kraaikamp and Bloemendal who, having developed

at Nijmegen methods for isolating 14S RNA preparations which include messenger molecules for αA_2 crystallin, have collaborated with Lane in Gurdon's laboratory at the University of Oxford.

Apparently in *Xenopus* oocytes 14S crystallin mRNA is translated with fidelity into αA_2 crystallin, which, like the crystallin made in calf lens cells, has an acetylated N terminal methionine residue. This finding suggests that an acetylating enzyme capable of acetylating N terminal amino-acids may have a widespread distribution in eukaryotic cells and, as the group so rightly say, it "lends support to the idea that the oocyte system may prove to be a generally useful microassay for eukaryotic mRNA molecules".

IONOSPHERE

Heat Probing at Arecibo

from a Correspondent

THERE has been a resurgence of interest recently in the use of radio waves in heating ionospheric electrons. Two groups, one at Boulder, Colorado, and the other at the Arecibo Observatory, have shown that ionospheric parameters can be deduced from heating experiments. Heating arises in the following way: an electron moving under the influence of the electric field of a radio wave may have its ordered motion destroyed by collision with a heavier ion or neutral particle. Energy is thus extracted from the wave and converted into random thermal energy of the electrons. Because electrons only give up a fraction of their energy in a single collision with a heavier particle there is a net increase in the temperature of the electrons. The plasma pressure increases and the plasma diffuses along the magnetic field lines until the pressure is equal to that of the surrounding plasma so that the heating is associated with a local decrease in plasma density.

An effect attributable to ionospheric heating was named the "Luxemburg effect" after the radio station on which it was first observed. This is a type of interference which is observed with powerful transmitters in which the modulation on one carrier frequency may be superimposed on another. The cross-modulation occurs when a radio wave is passing through a region where the electrons are being heated by a wave from another transmitter. This effect has been used in the past to determine electron densities and collision frequencies at low altitudes.

In 1970 researchers at Boulder using at 2 MW transmitter produced a detectable increase in temperature in the F region of the ionosphere at a height of

300 km. The team showed, with photometer measurements of 6300 Å airglow, that the heating transmitter produced a 30 per cent increase in electron temperature and these effects were observed within 30 s of the transmitter being switched on (*J. Geophys. Res.*, **75**, 6402; 1970). These experiments were carried out close to the F-layer critical frequency where maximum wave absorption and therefore maximum heating occur.

R. L. Showen of Rice University, Texas, has now shown, using a transmitter at Arecibo, that this heating technique may be extended to the lower ionosphere (*J. Geophys. Res.*, **77**, 1923; 1972). Using a 40 mHz transmitter with a peak power of 1 MW, he found that it is possible to double the electron temperature at a height of 90 km. Showen's experiment differs from the earlier experiments at Boulder in that the frequency used was well above the penetration frequency for the ionosphere and consequently there is very little attenuation of the wave; yet the electrons because of their small thermal capacity are significantly heated. The theory outlined by Showen indicates that a 5 ms pulse would increase the electron temperature by about 100 degrees.

The electron heating effect depends on electron collisions with neutral molecules and thus increases with decreasing altitude. But the rate at which the excess electron energy is dissipated is also dependent on collision frequency and below about 90 km the heating persists for only a few milliseconds, compared with tens of milliseconds at 100 km; it is consequently undetectable.

In the Arecibo experiment the change in electron temperature is determined by an incoherent backscatter technique. The backscatter from a radar pulse depends on the ratio of the electron to ion temperatures. If the ion temperature is assumed constant then a change in the backscattered power may be attributed to a change in the electron temperature. Simple theory shows that a 10 per cent increase in temperature reduces the backscattered power by about 5 per cent. Showen used pulses from a 430 mHz radar transmitted before and at intervals after the heating pulse to monitor the changes in electron temperature. Using heating pulses with durations between 0.5 and 5 ms, Showen determined collision frequency profiles in the altitude range 90–110 km and thermal relaxation times which had not been previously measured at these altitudes.

The Arecibo results and the earlier work at Boulder offer convincing evidence that ionospheric heating may be a valuable technique for probing the ionosphere over a wide range of altitudes.

DNA SYNTHESIS

Priming the Polymerase

from our Cell Biology Correspondent

PLAYING David is a risky business, for most giants are capable of putting up a deal more resistance than Goliath managed and either drive their challenges from the field or, what is perhaps even more humiliating, drive them to oblivion simply by not deigning to take notice. But, irrespective of the final outcome, few people are not enthralled by the sight of somebody trespassing on some giant's backyard only to throw a stone through his window. One cannot help wondering, for example, what will be the response to Keller's report, in the current issue of the *Proceedings of the National Academy of Sciences* (69, 1560; 1972), of experiments which prove that RNA primer molecules can initiate the synthesis of phage ϕ X174 DNA by mammalian as well as bacterial DNA polymerase.

The notion that the initiation of bacterial and phage DNA synthesis might be effected by an RNA primer molecule rather than a DNA primer stems from the observation, made by several groups, that the replication of *Escherichia coli* DNA and some coliphage DNAs seems to be tightly linked to the transcription of the DNA genome which is to be replicated. When transcription is prevented by one means or another DNA replication is inhibited.

One of the most intriguing interpretations of this finding, which was pointed out by Kornberg and his associates last year, is that transcription might be required because it might provide a short RNA chain, which, complementary to its template DNA strand, could act as a primer presenting a free 3' hydroxyl group onto which DNA polymerase could polymerize deoxynucleoside triphosphates. Not so many years ago such an unorthodox idea might well have been killed by ridicule had it ever appeared in print. But the discovery that RNA tumour virus particles contain an enzyme which is capable of using RNA molecules both as primers and as templates for the synthesis of complementary DNA so changed the climate of opinion that by last year the suggestion that an RNA might prime DNA replication seemed not at all unreasonable. Indeed it has now been verified unambiguously by Keller's elegant experiments with appropriate *in vitro* systems.

Keller found that if DNA polymerase II extracted from KB cells, which are of human origin, or if DNA polymerase I from the bacterium *Micrococcus luteus* is offered pure coliphage ϕ X174 DNA (single stranded and covalently circular molecules) as template, polymerization

of deoxynucleoside triphosphates into complementary DNA does not occur. When, however, DNA dependent RNA polymerase from *E. coli*, together with the four ribonucleoside triphosphates, are added, RNA and DNA synthesis begins and any interference in RNA synthesis results in a diminution of DNA synthesis.

In this most heterologous cell free system DNA synthesis could, however, be uncoupled from RNA synthesis if the DNA polymerases were offered as template DNA/RNA hybrids produced by preincubating the DNA with the RNA polymerase and its substrates. Reverse transcriptase from avian myeloblastosis virus particles could not substitute for DNA polymerase in either the coupled or uncoupled reactions. All these data suggest strongly that the DNA synthesis is being primed by an RNA primer, and Keller confirmed that the newly made DNA is linked to RNA by a 3':5' phosphodiester bond by physical and chemical tests. He then proceeded to show that the

product of the coupled RNA-DNA synthesis reaction is a double stranded nucleic acid comprising the template DNA chain hydrogen bonded to the product RNA-DNA chain by exposing the product to ribonuclease H from KB cells, which specifically digests the RNA moiety of RNA-DNA hybrids, and to *Neurospora* nuclease which degrades single but not double stranded RNA or DNA.

These experiments clearly prove that RNA chains can prime the initiation of DNA synthesis. To what extent prokaryotic and eukaryotic organisms avail themselves of this potential has yet to be determined, but as Keller points out the widespread distribution of RNAase H in eukaryotes and its occurrence in RNA tumour viruses suggest that RNA-DNA hybrid molecules may often turn up in animal cells and in bacteria. RNAase III might play the part of RNAase H. Clearly, Keller has struck a rich seam; it will be interesting to see who joins in the rush to exploit it and who ends up as chief proprietor.

Why Venus is Cloudy

IN next Monday's *Nature Physical Science* (July 3) J. T. Bartlett and G. E. Hunt put forward a suggestion to account for the difference in the degree of cloud cover in the atmospheres of the Earth and Venus. They suggest that, contrary to the suggestion of Goody and Robinson (*Astrophys. J.*, 146, 339; 1966), there may be no cloud free region at all over the surface of Venus.

Goody and Robinson's work depended on the assumption that a general upward motion in the atmosphere of Venus produced widespread cloud cover, just as cloudy regions on Earth are generally associated with areas of upward motion in the atmosphere; but these authors went on to conclude that this general upward movement must be countered by a small region of descending atmosphere on the cooler (dark) side of Venus. Such a cloud free region could, they argued, be too small to detect from Earth using present observational equipment.

On the basis of more recent evidence, including the direct measurements made by the Venera 7 probe, Bartlett and Hunt describe a two component model of the atmosphere in which a dense cloud layer in the troposphere covers a diffuse haze in the stratosphere.

It seems that such a model is consistent with the presence of a supersaturation of the cloud forming substance—whatever that may be—in the Venus atmosphere. Because of the decrease of pressure and temperature in the troposphere, this substance might exist as a vapour in the lower regions of the atmosphere but there will be a region

completely enclosing the planet in which the atmosphere is supersaturated with the cloud forming substance. Irregular mixing of the various components of the atmosphere will produce irregularities in the cloud bases; but a continuous cloud cover will exist, say Bartlett and Hunt, provided there is always some level in the troposphere above which the partial pressure of the cloud forming substance exceeds its saturation vapour pressure.

On Earth, those clouds which do form can be dispersed either because of precipitation or because they become mixed with unsaturated air. But if there were no efficient precipitation mechanism, as seems to be the case on Venus, the available pool of cloud forming material on the planet would continue to evaporate until the air in contact became saturated. Of the two precipitation mechanisms operating on Earth one depends on the coalescence of water droplets and the other on the presence of supercooled liquid together with a few solid particles which act as seeds.

According to Bartlett and Hunt, there is no reason to believe either that an appropriate distribution of droplet sizes exists on Venus for the first mechanism to operate, or that the cloud forming substance—whatever it may be—exists within the critical temperature range close to its freezing point necessary for the second mechanism to work.

The cloud forming process on Venus, it seems, is fundamentally different from that of Earth, where an efficient rain making mechanism ensures only partial cloud cover.

Value of Objective Examinations

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In some cases the traditional essay examination question is being superseded by more objective tests, requiring straightforward answers. If administered carefully these can provide a reliable means of assessment, but much research remains to be done.

THE widespread criticism of conventional examinations has focused chiefly on two aspects, their unreliability—different examiners often having very different views of the value of the same answer—and the narrow but often ill-defined and artificial range of abilities which are tested. In many subjects the introduction of objective examinations, which generally require only a tick to one of several suggested answers, could make an obvious and direct contribution to meeting the first criticism, and indirectly can do much to answer the second.

As far as reliability is concerned, objective examinations could make two contributions. Obviously marking can be completely reliable, and although there is a subjective element in the setting of questions and establishing and applying criteria of marking, this can be reduced by debate within the departments. Marking procedures for traditional examinations, however, attract little discussion and it is probably rare that there is much general agreement on broad criteria of judgment.

The other contribution concerns the "division of labour" between different objectives which objective examinations impose. With traditional methods, examiners can easily be confused about the relative merits of answers which are detailed and comprehensive compared with those which are thinner but more imaginative. Students can be more certain about what is expected of them in objective examinations, and this could be a significant contribution to reliability.

This "division of labour" can also be the beginning of a reappraisal of the objectives of a course of study, and lead to the introduction of forms of assessment which are associated with different objectives. This in turn can lead to a diagnostic approach which gives the student a profile of his abilities which is more individual and meaningful than the over-generalizations involved in overall unidimensional assessments of "academic worth". The introduction of objective examinations, however, may have no such beneficial effects. Before looking more closely at the advantages and disadvantages of objective tests I shall discuss the way in which they are constructed and the uses to which they can be put.

Construction

The development of intelligence tests at the beginning of the present century opened the way for a more scientific approach to the measurement of human abilities. Coupled with the growing awareness of how unreliable essay examinations could be and of a need for standards of comparison between many educational institutions in America, this led to the development of objective examinations. At first there was much opposition, but when teachers had gained experience of them, their fears were somewhat lulled by the advantages, especially with regard to administration. In

Britain in the past few years objective examinations have found increasing applications, primarily in medicine and science, but they are being more widely used in schools. Fears about objective tests seem to arise from too little or too much experience of them. Guilbert and Rousse-Lacordaire¹ found that teachers with little or no experience of multiple choice medical examinations were often violently opposed to them, whereas those with experience were generally in favour. Criticism in America centres around a tendency for these tests to dominate not only assessment but the learning process as well.

The apparent change in attitude after some experience of these examinations may be related to the way in which they are constructed. Whereas the setting of essay questions rarely involves any sustained discussion among staff about their educational objectives, even though it might be one of the few occasions when such issues are raised, with objective tests discussion has to be specifically diagnostic and can be educationally rewarding.

Ideally, anyone wanting to use objective tests seriously for assessment should attend a short course of instruction. But experience with more simple tests used only for feedback to staff and students can be a good basis for developing more technically respectable tests for use as part of the general assessment. The Educational Testing Service (ETS) of Princeton, New Jersey, produces a series of booklets about its tests and how teachers can construct their own. The National Foundation for Educational Research in England and Wales, has also developed a practical manual². Several fuller discussions have been published³⁻⁵. Only Hubbard and Clemans⁶ deal particularly with higher education and this is focused on medicine, but the general principles are much the same at all levels.

In the construction of objective examinations there is first the planning stage. The emphasis used to be on getting a correct sample of the content of the course, but although this is still a vital part of the planning, more importance is now attached to a detailed analysis of what the tester is trying to do. This raises much broader questions of educational aims and even educational philosophy. The first volume of Bloom's *Taxonomy of Educational Objectives*⁷ in which he classifies educational objectives under six headings—knowledge, comprehension, application, analysis, synthesis and evaluation—has been a useful basis for thinking about objectives. It is not always easy to categorize the items, as the questions are called, under these particular headings, but Stauffer⁸ suggests that there can be a high level of agreement between different judges^{9,10}. Teamwork is an important feature of the construction of tests, and even if a particular teacher wants to construct them purely for his own purposes it is worth discussing them with colleagues. The participation of committees not only helps to produce better tests and reduce subjectivism, but provides a productive setting for the discussion of educational problems. But although discussion may help to define educational objectives, the tests (or construction requirements) should not themselves help to determine objectives but should seek to reflect them as faithfully as possible.

After the planning comes the writing of the test items, usually by the teaching staff, ideally with the help of specialists. There is a great deal to be learned about this process and experience is very useful. Ebel¹¹ suggests that propositions about knowledge which can be used for objective test items should meet at least four requirements.

(1) They must be worded as accurately and unambiguously as the precision of knowledge and language allow in a reasonably concise statement. (2) They must be acceptable as established truths by a preponderance of experts in the field. (3) They must be regarded as the propositions most worthy of knowing and remembering by a preponderance of experts in the field. (4) They must express principles and ideas not generally known by those who have not studied the field. Clearly it is not easy to write test items, but the value of doing so is not confined purely to producing good tests.

The next stage is to try out the test. There are two inter-related questions here: how well does the test work as a whole, and how well does each item work. The test therefore should be tried out on a group of students and an item analysis carried out. This can be done using short cut methods or, if computing facilities are available, correlation methods can be used. Usually the aim is to achieve an average of about 60 per cent correct responses with very few at the extremes. The discriminating power of each item might be assessed in a simple way of looking at how many of the top 25 per cent of students on the whole test gave the correct answer and how many of the bottom 25 per cent did so. Items for which the high group score considerably higher than the lower group are considered to be usefully discriminating; if there is not this difference the items should be examined carefully, for the test might be measuring something quite different or it might be ambiguous or misleading. Generally the tests are arranged so that most students attempt all questions and speed is not an important factor. The number of questions in the test depends very much on the time available and the homogeneity of the general population of possible questions to be asked on that particular course. Principles of sampling are followed so that the more homogeneous the population the fewer the questions needed to give a particular level of reliability. Sometimes it is worth doing an item analysis for each of the main objectives or mental abilities separately, since one particular large group might dominate the analysis and items might be discarded chiefly because they belong to a small group¹³.

In the assembly stage the test items are selected in terms of difficulty, discrimination, coverage of objectives and content. Sometimes decisions have to be made about the use of new types of items and whether they are only to be used experimentally.

The next stage is usually to judge the validity of the tests. If they are used primarily for selection or prediction, validation is simply a case of finding out how well they work by following up those who have been given the tests. When the tests are used for assessing students and teaching, the question is more complicated. Teachers' opinions about how well a test fulfils its aims are useful, but there are difficulties because they may base their judgment on different criteria.

The final stage might be to interpret the results. This involves establishing the norms, and in the report *Technical Recommendations for Achievement Tests*¹³, all the information which should go with a published test is described, indicating what can and cannot be inferred from scores. When tests are prepared by teachers, local norms can be important in comparisons of groups who have been taught or selected differently, as well as for the interpretation of individual scores. Sophisticated computer analyses are now becoming more common^{14,15}.

Types of Objective Examinations

There are now many types of objective examinations and, although the weighting of different aspects of achievement and the writing of good items is more important than the choice of any particular type of item, some types are more suitable than others for particular purposes. Again, for anyone thinking of constructing objective examinations or

using them in a serious way, it would be essential to look at the different types in detail in the references mentioned earlier. Ebel¹¹ considers that the most commonly used types fall under four headings—multiple choice, true-false, matching and classification.

The multiple-choice objective examinations, in which a question or incomplete statement is followed by four or five suggested answers or completions from which the students select the best, is the most common. Good items are not easy to write because the distractors, incorrect responses, must all be plausible or the correct answer is likely to be guessed. Nevertheless, if they are too plausible it might be felt that in some circumstances there is merit in the student choosing them. Not all such questions have to depend on recognition. If one of the alternatives is "none of the above" and is sometimes the correct answer the student must then think of answers which are not written down. A slight variation is to ask the student to mark the choice which does not apply to a certain situation. Multiple-choice is a very adaptable type of item and shows good discrimination. It has been shown to be as reliable as a true-false type of test when using only half the number of items.

With the matching type, several items are to be associated with a group of possible responses. For example, the authors of three novels might have to be selected from a list of names. This type of item can be rather more compact but often more difficult to write than the multiple-choice question. A slight variation of this is what Hubbard and Clemans call "a four choice association".

The classification type of item is similar to matching in that a single set of responses is used but in this case it is applied to many similar situations. Ebel¹¹ gives an example of a test for which the directions are: "In the following items you are to express the effects of exercise on various body processes and substances. Assume that the organism undergoes no change except those due to exercise. For each item blacken the answer space". There followed three numbered responses—(1) the effect of exercise is to increase the quality described in the item, (2) it decreases it, (3) it has no appreciable effect or an unpredictable effect. Then follows a list of stimulus situations like rate of heart beat, blood pressure, amount of glucose in the blood, and after each are the numbers 1, 2, 3, one of which is to be blackened. An advantage of this type of question is that it can focus attention on a single but complex topic and thinking around this topic can stimulate a type of memory which comes from understanding rather than the rote memory which is often all that can operate where there are a large number of completely discrete questions. This factor operates even more clearly in some of the newer type of medical objective examinations where the questions are fitted into what is very like a programmed learning text with branches to be followed depending on the answers that the students give.

The true-false type of item is less common now than previously because of problems with guessing. Ebel¹¹ says this limitation can be overcome not by introducing a conventional guessing correction, but by a special scoring procedure based on the examinee's indication of how much he was guessing on each answer. Personality, however, may be an important factor in the accuracy of the indication.

Hubbard and Clemans⁶ give several interesting variations on items of the multiple-choice type. That which they call relationship analysis is an attempt to bring in reasoning ability but it is not clear that it would necessarily do so. Walton and Drewery¹⁶ give an example with an analysis of the results (top of following page).

Often rather precise rote memory of quantities is required, but Hubbard and Clemans suggest that where possible this should be avoided by using questions designed to compare different statements in a quantitative sense.

Directions

Circle 1. If both statements are <u>true</u> and the second is the correct explanation of the first	Students selecting each alternative (No.)	Correct (%)	Validity index
	1 2 3 4 5		
	2 3 94 1 6	85	0.43
Circle 2. If both statements are true but the second is not a correct explanation of the first	Comment This is a satisfactory item in that although easy it correlates well with the whole test		
Circle 3. If the <u>first</u> statement is true but the <u>second</u> false			
Circle 4. If the <u>first</u> statement is <u>false</u> but the second is <u>true</u>			
Circle 5. If both first and second statements are false			
It is essential to determine the intellectual abilities of an emotionally disturbed school-aged child	because	neurotic children are usually of low intelligence.	

There is no doubt that there is plenty of scope for devising new types of objective questions, and their integration into programmed learning (as is already happening in medicine) will be an important factor, but the evidence that different forms of test will test different abilities is by no means clear and this suggests that research is not taking adequate account of the complexity of the subject.

Advantages and Disadvantages

Unfortunately there seems to be very little research in progress on the general validity of objective examinations. There is a little in medicine, however. In 1952 the National Board of Medical Examiners introduced multiple-choice type questions into their qualifying examinations, after undertaking a study in cooperation with the ETS¹⁷. In this study, parts of the regular examinations in pharmacology and internal medicine were changed to multiple-choice form and the other half were left in the usual essay form. The essays accounted for two hours and the multiple-choice accounted for one and a half hours of the total time provided for each of the two subjects. For both medicine and pharmacology the correlation of multiple-choice scores and ratings of staff on the students was significantly greater than either of the correlations involving essay test grades. They concluded that if the staff ratings could be accepted as a suitable criterion the multiple-choice tests were more valid measurements of student proficiency than essay examinations.

In Britain correlations of essay and objective examinations with total marks in pathology and bacteriology have suggested that objective examinations have distinct advantages. Anderson, Dykes and Lennox¹⁸ give the figures shown in Table 1.

Of course, the reliability of the examiners might contribute to this higher correlation, but reducing sampling error may well be just as important. Hubbard and Clemans⁶ suggest that a three-hour paper containing 300 items would cover the same amount of information as could be covered in eighteen essay questions. Since the essay questions would tend to cluster the information on certain topics the objective examinations would spread the sampling over a much wider field.

Objective examinations avoid contamination by what might be considered irrelevant abilities. Furneaux¹⁹ and others have shown that a general ability to pass examina-

tions is responsible for much of the variance between students, and speed is an important factor. But with objective examinations speed and verbal fluency would be less important. Of course, if it is intended to test speed, then objective examinations can be adapted to this, but with essay examinations it is very difficult to eliminate speed.

Within important limitations objective examinations can contribute to counselling, evaluation of teaching and improving learning. Their better coverage, ease of administration and standardization make it possible to assess particular strengths and weaknesses over the whole range of a course. In other words, a more diagnostic approach can be adopted and this can be particularly useful as an aid to teaching. In the United States there is now an increasing use of tests at the beginning of the year to help in planning courses and to fill important gaps in students' knowledge. Frequent testing along the lines of that which is built into programmed learning can give rapid, useful feedback to both staff and students. But it can be an encouragement to an approach to learning which is geared simply to getting high marks and over-tested students can develop attitudes which are difficult to change. On the other hand, criticism of students for wanting high marks can be a criticism of the examining system which rewards what it should not be rewarding. Unfortunately it still seems to be true that the ease with which objective examinations measure acquisition of information means that this is given undue prominence. But, on the other hand, there is evidence²⁰ that, in physics at least, university essay examinations too can be dominated by the ability to recall facts. But while there is little doubt that objective examinations at pre-university level can test much more than factual information, any attempt at a higher level to measure more complex abilities will run into two difficulties. One is that if judgment is required, then if the question is not to be particularly facile it is difficult to avoid a situation where alternative judgments do not have some merit, and this is not often allowed for in the scoring system (see ref. 21). The British Postgraduate Medical Federation, however, is now deliberately exploiting this feature to test "judgment". The other difficulty is that answering complex questions often involves stages, and while it may be possible to test these separately without revealing the answer or altering what is assessed this is difficult and where it is not done the final correct step can be over-weighted. If many questions are still asked this may not be a serious problem, but, of course, the more complex the question becomes, the fewer the questions that can be put into any one examination.

It is often said that objective examinations cannot test the student's ability to organize his work, and, of course, this is true, but it is perhaps more important than is sometimes realized, for organizing a long essay answer can involve developing a deeper understanding of the subject and this cannot be done in the seconds or minutes it takes to answer objective questions. Much work is being done on developing relatively objective tests of creative ability, but there is still far to go, and although there may be no harm in experiments by individual teachers, it is unlikely that great progress will be made in particular subjects without very serious research by experienced psychologists.

The contribution which objective examinations make to education depends very much on whether their undoubted virtues are exploited for convenience, as a technique which becomes an end in itself or in more imaginative ways. The danger is that the large investment of time and expertise will lead to both over-use and a reluctance to change. Some knowledge is quickly out of date, but it may prove to be only too easy to put off the task of revising large item banks.

On the other hand, there is little doubt that the developments of objective examinations can, perhaps more than anything else, focus attention on objectives, and this could

Table 1 Correlation of Marks for Essay and Objective Papers in MB Examinations with the Final Total for the Examination

Type of paper	1963	1964
Essay	0.69	0.56
Objective	0.82	0.82

lead to a clearer idea of the functions both of objective examinations and of more open forms of assessment. Courses, moreover, are becoming notoriously over-crowded and students cannot be expected to have more than an acquaintance with certain areas without jeopardizing valuable study in depth. Objective examinations may well have an important function here even in fields where they have not been used much before. There are signs now of a healthy openness to new methods of examining and the development of objective examinations can certainly make a valuable contribution to this—provided that they are used to introduce more diversity and flexibility and not merely to lighten the load of marking.

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Measurement from Gauss to Whitehead

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This article is a summary of an address by Professor Temple to the British Society for the History of Mathematics at its first meeting on December 17, 1971.

THE geometrical theory of measurement is dominated by Riemann's famous *Habilitationschrift*, but it is necessary to see this great work against the background of geometrical research in the first half of the nineteenth century. Three different lines of thought converged to unite in Riemann and then to develop in three stages.

First, the famous "parallel postulate" of Euclid and his use of congruence as a method of proof issued a challenge to geometers which was met in the nineteenth century by the creation of metageometry and by the theory of measurement. Gauss, Lobatchewsky and Bolyai invented non-Euclidean geometries which dispensed with the parallel postulate while Beltrami established the internal consistency of these new geometries by constructing a model in Euclidean space.

Second, the concept of congruence was enriched by the work of Gauss on the specific curvature and the applicability of surfaces, and this enabled Riemann to formulate the idea of "free mobility" as a characteristic of rigid body motion.

Third, the theory of transversals, as developed by French geometers, led to the principles of projection and of the continuity of geometrical properties, and to the introduction of imaginary points such as the "circular points at infinity" and the recognition of the projective invariance of the anharmonic ratio.

Riemann's researches contain, in an informal, suggestive and enormously influential form, not only a sketch of "spherical geometry" but also the concepts of a manifold, of general coordinate systems, of the necessarily Pythagorean form for

the infinitesimal line element, of geodetic coordinates and of the famous four-index symbols.

The Legacy of Riemann

The verdict of Veronese, quoted with approval by Bertrand Russell, that "Riemann ist in seiner Definition des Begriffs Grösse dunkel", may justify the following benign interpretation of Riemann's obscure and suggestive phrases.

A one dimensional manifold, M_1 , is given by a continuous mapping

$$f: M_1 \rightarrow R$$

of the elements of M_1 (points M_0) into the real numbers R , such that the function f is not constant in any open set in M_1 . An n -dimensional manifold, M_n , is given inductively by a continuous mapping,

$$g: M_n \rightarrow R$$

of the elements of M_n ($n-1$ dimensional manifolds M_{n-1}) into the real numbers R .

From this concept of a continuous manifold, M_n , Riemann is able to introduce a system of coordinates $x_1, x_2, \dots, x_n$.

Having thus despatched the problem of defining a manifold, its dimensions, and coordinate systems, Riemann turns to the concept of "distance". Here he assumes that with any two points, A, P , of a manifold there is associated a number $\delta(A, P)$, to be called the distance between A and P , and that the equation of the sphere, of centre A and radius c , for example, $\delta(A, P) = c$, can also be written in the form $f(P) = \text{constant}$, where f is a non-negative function of the coordinates $x_1, x_2, \dots, x_n$ of P , with continuous second derivatives, which attains its minimum value, namely zero, when $P = A$. Then, in general, the leading terms in the expansion of f must form a positive, definite quadratic function

$$Q = \sum_{j,k} g_{jk}(x_j - a_j)(x_k - a_k)$$

Assuming that the distance function $\delta(A, P)$ is approximately

a linear function of the co-ordinate distances, $\{x_k - a_k\}$, when δ is small, Riemann concludes that to the same approximation, Q has the form $\lambda(P)\delta^2(A, P)$ and thus establishes the Pythagorean expression for the distance ds between neighbouring points in the form

$$ds^2 = \sum_{j,k} g_{jk} dh_j dx_k$$

From the expression for the "infinitesimal" line element ds , Riemann could introduce "geodetic coordinates", $y_1, y_2, \dots, y_n$, and hence expresses the terms of the second and fourth order in the expression for ds^2 in the form

$$ds^2 = \sum_{jk} dy_k^2 - \frac{1}{2} \sum_{ijrs} R_{ijrs} (y_i dy_j - y_j dy_i) (y_r dy_s - y_s dy_r)$$

and we may well believe that he identified the co-efficients R_{ijrs} with the four-index symbols which he later discovered in his researches on the solutions of the equation for the conduction of heat.

Three Questions

After Riemann, the theory of measurement is the history of attempts to answer three questions of deepening abstraction and difficulty. How are distances compared by the transport of rigid bodies, or by constructions based only on straight lines, and how can a straight line and a point be characterized?

The answer to the first question was the metrical theory of congruence, attempted first by Helmholtz with the concept of free mobility and given in definitive form by Lie in his theory of finite groups. Helmholtz's interest in the foundations of geometry arose from his work on the physiology of vision, and his researches exemplify Hermann Weyl's dictum that "physics is too difficult for physicists", by which aphorism I presume he meant that the solution of physical problems may require very sophisticated and mathematical techniques. Lie's theory of continuous groups of transformations was not available when Helmholtz, stimulated by Riemann's theory of distance, undertook to deduce the expression for the line element from the axioms of congruence. In fact, Helmholtz's proofs are far from rigid, but they provided the groundwork for Lie's investigations.

Helmholtz's researches are based on four axioms which can be expressed as follows. First, the points of physical space form an n -dimensional manifold, that is, they can be mapped into a Euclidean space of n -dimensions; second, in any motion of a rigid body there is a numerical function of the $2n$ co-ordinates of any point there which remains invariant; third, a rigid body can be moved so as to transfer any point to any other point; and, fourth, if $(n-1)$ points of a rigid body are fixed, then there is a motion of the body in which each point describes a closed curve.

From these axioms Helmholtz attempted to establish that if ds is the element of distance between neighbouring points with coordinates $(x, y, z) + (dx, dy, dz)$, then ds^2 is a homogenous quadratic function of dx, dy, dz . It seems generally agreed that his argument is unconvincing, and Lie devoted thirty-four pages to a criticism followed by his own two solutions of the "Riemann-Helmholtz" problem, starting from two different characterizations of the motion of rigid bodies in a three-dimensional manifold with a coordinate system, x, y, z .

Lie's Solutions

Both solutions depend on the expression of our physical experience in such a form that the possible motions of a rigid body must form a continuous group of transformations. Once this fundamental fact had been stated explicitly, Lie was in a position to use all the powerful machinery of his theory of continuous groups.

Lie's first solution depends on his definition of "free mobility in the infinitesimal" in the form that if a point P and an arbitrary real line element passing through P are fixed, then continuous motion is still possible, but if in addition another

distinct real line element passing through P is also fixed then no continuous motion is possible. This is almost all that Lie required in order to prove that the coordinates of the space can be chosen so that the group of rigid body motions is either (i) the group of translations and rotations in Euclidean space, or (ii) the six-parameter group of all real projective transformations which leave invariant either the imaginary surface

$$x^2 + y^2 + z^2 + 1 = 0$$

or the real surface

$$x^2 + y^2 + z^2 = 1$$

In other words, the geometry of the space in which rigid bodies have free mobility in the infinitesimal must be either Euclidean or one of the species of so-called non-Euclidean geometry.

Lie's second solution assumes that when an arbitrary real point (a, b, c) is fixed, the real points (ξ, η, ζ) into which it is possible to move an arbitrary point (x, y, z) satisfy a real equation of the form $W(a, b, c; x, y, z; \xi, \eta, \zeta) = 0$ and the inequalities $\xi \neq a, \eta \neq b, \zeta \neq c$, the function W being continuous in ξ, η, ζ . Once again, Lie showed that this assumption is sufficient to show that the space of a rigid body motion is either Euclidean or non-Euclidean.

Lie's theory of continuous groups inspired Poincaré to examine the problem of congruence in a plane, regarded as a two-dimensional manifold in which the position of a uniplanar rigid figure is completely specified by three conditions. Poincaré showed that even this very general condition is sufficient to limit two possible geometries, namely Euclidean and non-Euclidean geometries, and two somewhat bizarre geometries in which uniplanar rigid figures can still move although two distinct points are fixed.

The classical or non-relativistic problem of congruence envisaged a rigid body at rest in two different positions, and a transformation from one such position to another. But the question was radically changed by the special and general theories of relativity. In the special theory of relativity, the same rigid body (or at least the same rigid measuring rod or clock) is envisaged in two different frames of reference, which may be in relative motion. As a result, we have to deal with a manifold in which there are three space-like coordinates, x, y, z , and a further timelike coordinate, t . The distance between two points in space is now to be replaced by the interval between two events with coordinates (x_1, y_1, z_1, t_1) and (x_2, y_2, z_2, t_2) . In the special theory of relativity, this interval is given by the expression

$$c^2(t_1 - t_2)^2 - (x_1 - x_2)^2 - (y_1 - y_2)^2 - (z_1 - z_2)^2$$

where c is the velocity of propagation of light. The numerous axiomatic approaches to the special theory of relativity attempt to characterize the group G of transformations which relate the space-time measurements made by rigid rods and clocks in uniform motion, and are usually based on the postulate of Euclidean geometry of rigid bodies in relative rest. It is also postulated that the group G leaves invariant the linear equations which purport to describe the motion of a particle free from external influence. The study of the special theory of relativity had compelled geometers to consider manifolds in which the necessarily positive distance between two points was replaced by an interval whose square could be positive, negative or zero. But it was the impact of the general theory of relativity which had the more profound influence on the theory of measurement. In the general theory, the expression for the interval ds between two neighbouring points with coordinates (x_1, x_2, x_3, x_4) and $(x_1 + dx_1, x_2 + dx_2, x_3 + dx_3, x_4 + dx_4)$ has the form

$$ds = \sqrt{\sum g_{\mu\nu} dx^\mu dx^\nu}$$

and the whole of the theory is based on the infinitesimal or local properties of the manifold which are implicit in this

expression. This is the type of metageometry which was envisaged by Riemann and which deservedly bears his name. In this space-time manifold the first problem is to compare the spatial and temporal measurements made by two observers instantaneously located at the same event, but in relative motion, and for this purpose an extension of the Helmholtz-Lie group theory is entirely adequate. It is, moreover, more difficult to make similar comparisons for two observers located at different events, even when these are neighbouring events.

The problem is to compare two vectors U and V located at different events P and Q . In the special theory there is a definite meaning in saying that U and V are equal and parallel, namely that their corresponding space-time components are each equal in the system of coordinates wherein the interval between distant events has the form

$$c^2(t_1 - t_2)^2 - (x_1 - x_2)^2 - (y_1 - y_2)^2 = (z_1 - z_2)^2$$

But in general theory there is no such general property of teleparallelism.

For the purposes of measurement, however, all that is necessary is to study the transport of vectors (rods and clocks) along prescribed tracts in space-time, that is, to develop a theory of the parallel displacement of vectors along prescribed curves in a Riemannian manifold. The first solution to this problem was given by Levi-Civita by imagining the Riemannian manifold to be embedded in a Euclidean space of sufficiently many dimensions.

The analytic expression of parallel displacement now gave a geometrical significance to the three index symbols, introduced long before by Christoffel, and the dependence of parallel displacement on the track specifying the displacement also gave a new meaning to the four index symbols which Riemann had invented in studying the equation for the conduction of heat in curvilinear coordinates.

There remains, however, the fundamental problem of why there should exist a quadratic form in the infinitesimals dn^1, dn^2, dn^3, dn^4 for the square of the interval ds . A question of this difficulty naturally attracted the attention of Weyl, who furnished a solution which all admire even if few claim to understand it.

Projective Theory of Distance

The researches just described summarize very briefly the attempts which have been made to find out how distances are compared by the transport of rigid bodies or by clocks. These researches comprise the metrical theory of measurement. The next set of researches which I shall discuss constitute the projective theory of distance, which is based solely on constructions made by straight lines and which dispense entirely with the initial assumption of free mobility.

The two great names associated with this theory are undoubtedly those of Cayley and of von Staudt. Cayley's great discovery in the projective theory of distance is somewhat obscured in his famous paper, partly by the now antiquated language of the theory of quantics (homogenous polynomials), and partly by his own facility in algebraic manipulation. The essence of Cayley's discovery can, however, be stated as follows. In Euclidean space, if p, q, r are three collinear points and if q is between p and r then the Euclidean distances between p and q , and between q and r , when added together are equal to the Euclidean distance between p and r . Cayley showed that there are other functions of these coordinates, which we may call Cayley distances, with precisely the same additive properties and that any such Cayley distance function is related to a fixed quadric in the plane which he called the Absolute. Although Cayley never seems to have contemplated seriously the possibility of the objective existence of non-Euclidean space it was left to Klein to start from Cayley's discovery of the Absolute, and to characterize the continuous group of congruent transformations in non-Euclidean space as the projective transformations which leave invariant the absolute quadric.

The whole of the metric theory of measurement from Riemann and Helmholtz to Weyl and Eddington is based on the assumption that it is possible to fix positions in space or in space-time by means of coordinates. But how can coordinates be introduced without using some method of measurement—bearing in mind that the object of all these theories was, of course, to justify the concept of measurement? All these theories therefore seem to revolve in a circle, but the difficulty was resolved by the researches of von Staudt, who showed as early as 1846 that it is possible to introduce coordinates without any appeal to quantitative measurement.

Before von Staudt, the anharmonic or cross ratio of two pairs of points (A, B, P, Q) had been defined in terms of the length of the segments AP, PB, AQ, QB , and such a collection of four points had been described as a harmonic range if the metrical cross ratio $(AP/PB) \div (AQ/QB)$ was equal to -1 . Von Staudt's innovation was to give a non-metrical projective definition of the harmonic range by a construction due to Möbius based on the complete quadrilateral. Having thus given a projective characterization of the harmonic range, von Staudt was then able to define a projective relation between sets of points on two distinct lines to be one which transformed the harmonic range on one line into a harmonic range on the other. According to this definition it is easily verified that if two pairs of points $(A, B; P, Q)$ form a harmonic range, then so also do the two pairs $(A, B; Q, P)$ and $(B, A; P, Q)$. By an ingenious and elaborate construction based on the "Möbius net" von Staudt was able to use this property to give a projective definition of coordinates on a straight line in a plane and in space. It was now possible to give a projective definition of a quadric, and to bring together Cayley's definition of distance and von Staudt's definition in terms of the anharmonic ratio of two points and of the other two points in which the line joining them intersects a fixed quadric—the Absolute.

Topological Theory

Finally, there is what may be called the topological theory of measurement. Although the projective theory had dispensed with the concept of distance as a fundamental and undefined entity, it had been obliged to start with the straight line and the point as fundamental undefined elements. The topological theory had as its ambitious aims to give a characterization of these geometrical elements. The first attempts in this direction seem to have been due to Menger who gave a definition of a point as the limit of a convergent sequence of nested bodies lying one inside the other like a sequence of Chinese boxes. This theory was developed independently by A. N. Whitehead in his method of extensive abstraction, which was applied originally to give a characterization of the events in the special theory of relativity. Although Whitehead's researches in this direction have found few followers, perhaps it is not too fantastic to see in his work the germ of the concept of "filters", which played such an important part in topology after its introduction by Henri Cartan.

In his first version of the method of extensive abstraction Whitehead based everything on the relation of inclusion, by which one body was included in another. But later, in his Gifford lectures, he adopted de Laguna's relation of "extensive connexion", by the aid of which he attempted to give a topological characterization of convex bodies. He was then able to define a straight line joining two points P and Q as the smallest convex body covering both P and Q .

If this ambitious programme had succeeded it would have drastically simplified the problem of the metrization of topological spaces, and would have reduced topology to projective geometry, thus completing the work of von Staudt, who had reduced projective geometry to metric geometry. The reception accorded to Whitehead's ideas may perhaps be adequately summarized in a phrase of H. G. Wells—"the ironic silence which succeeds a great debate".

Nature of Haem-Haem Interaction

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Haem-haem interaction consists of a change of tension at the haems, caused by a transition between two alternative quaternary structures of the protein. Dr Perutz describes how spin changes that accompany reaction with ligands alter the oxygen affinity of the haems.

In my recent paper on the stereochemistry of the cooperative effects of haemoglobin, I proposed that haem-haem interaction arises from a reversible transition between two states which differ both in the tertiary structure of the α and β subunits (t or r) and in their quaternary arrangement within the tetramer (T or R)¹. In the oxy or r form, the iron is low spin, the haem group is approximately planar and the haem-linked nitrogen of histidine F8 lies at about 2.0 Å from the plane of the porphyrin ring. In the deoxy or t form, on the other hand, the iron is high spin. The consequent lengthening of the iron nitrogen bonds displaces the iron atom and the nitrogen of the haem-linked histidine from the plane of the ring by about 0.75 and 2.9 Å respectively.

It now appears that there exists a reciprocal relationship between the positions of the iron atoms and the haem-linked histidines relative to the plane of the porphyrin ring on the one hand, and the quaternary structure of the haemoglobin molecule on the other. While it was proposed earlier that changes in the spin state of the haems trigger a transition between the two quaternary structures, I now find that the transition in turn alters the spin state of the haems. The T structure is literally tense (in Wyman and Monod's sense) in that it puts constraints on the haem, while in the R structure the haems are as relaxed as the free subunits. The constraints in the T structure raise the spin state of the haems, which diminishes their oxygen affinity by opposing the change to the low spin state. The results presented here and in the recent paper on haemoglobin M Milwaukee also show that in the absence of a change in quaternary structure, the combination of one haem with ligand produces no change in spin state in neighbouring haems. Without a change in quaternary structure therefore haem-haem interaction is absent.

The results lead to an interpretation of the changes in the hyperfine shifted proton resonances in the ferric haems that have been observed on dissociation of ligands from the ferrous haems in haemoglobin M Milwaukee. They also allow a physical interpretation of the variations in oxygen affinities of different haemoglobins and lead to the prediction of an inverse relationship between oxygen affinity and paramagnetic susceptibility of the deoxy forms. On this basis changes observed in the hyperfine shifted proton resonances of the ferrous haems of modified and mutant haemoglobins can be correlated with their oxygen affinities.

Interactions between Ferrous and Ferric Haems

In aquomethaemoglobin (metHb) the iron atoms take up positions intermediate between their out of plane positions in

deoxyhaemoglobin and in-plane positions in oxyhaemoglobin (Fe lies 0.3 Å from the plane in the R form¹). This also applies to the closely related methaemoglobin M Milwaukee, with the consequence that the abnormal subunits can take up either one of the two alternative tertiary structures without undergoing any chemical reaction, but depending merely on the quaternary structure of the tetramer. Their transition from the r to the t structure is accompanied by a change in the optical absorption band at 620 nm to lower intensity and longer wavelength, indicative of a transition to a state of higher spin². I now wish to report similar changes in metHb A. In order to observe these changes, I added less than one molar equivalent of reducer to solutions of metHb, and then filled each partially reduced solution into a pair of matched and stoppered cuvettes, bubbled CO through one of them, and recorded the difference spectrum. The results show that the absorption bands of the ferric subunits at 630 and 1,000 nm both change to lower amplitude and longer wavelength on loss of CO from the ferrous subunits. This corresponds to a transition of the ferric haems to a state of higher spin.

Experimental Methods and Results

To observe spectral changes in aquomet subunits against a background of change due to the ferrous subunits combining with CO, we have to select absorption bands in regions where neither deoxy nor CO haemoglobin show maxima or minima; the only ones that are suitable lie at 630 and 1,000 nm. Stripped human metHb was prepared by oxidation of a solution of oxyHb with a three-fold molar excess of ferricyanide, followed by passage through a 'Sephadex G-25' column equilibrated with 0.1 M NaCl. The solution was then brought to a concentration of 3.8 mM tetramer/l. by pressure dialysis. For spectroscopy it was diluted 100 times with 0.05 M *bis* Tris and 0.1 M NaCl of pH 6.5 or 7.0, and either 2,3-diphosphoglycerate (2,3-DPG) was added to a concentration of 200 μ M or inositolhexaphosphate (IHP) to a concentration of 60 μ M. This solution was divided into several portions of 7 ml. each and deoxygenated in a nitrogen-filled glove box. Na₂S₂O₄ was dissolved in the same buffer and neutralized with NaOH, also in the glove box. To each portion of the 38 μ M metHb solution, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.2 μ mol of Na₂S₂O₄ were added, thus producing mixtures of metHb and increasing proportions of deoxyHb. Each portion was put into a pair of matched and stoppered cuvettes and CO was bubbled through one of them, thus converting the deoxy portion of the mixture to HbCO, but leaving the metHb chemically unaltered.

The spectra and difference spectra of each pair of solutions were recorded on a 'Cary 14' spectrophotometer. Fig. 1 shows the uncorrected difference spectra for various percentages of reduction of the original metHb solution in the presence of 2,3-DPG. The difference spectrum of 100% deoxy minus 100% CO, shown uppermost, is a smooth curve. On the other hand, the difference spectra of the hybrid mixtures show minima at about 630 nm, coinciding with the maximum of the metHb absorption band, and maxima at 645–650 nm. These difference spectra are, of course, composite, being made up of the deoxy-CO difference plus the change in the metHb band. An attempt was made to subtract the former from the latter by a procedure described in the caption, giving the curves of Fig. 1b. These show that the amplitude of the

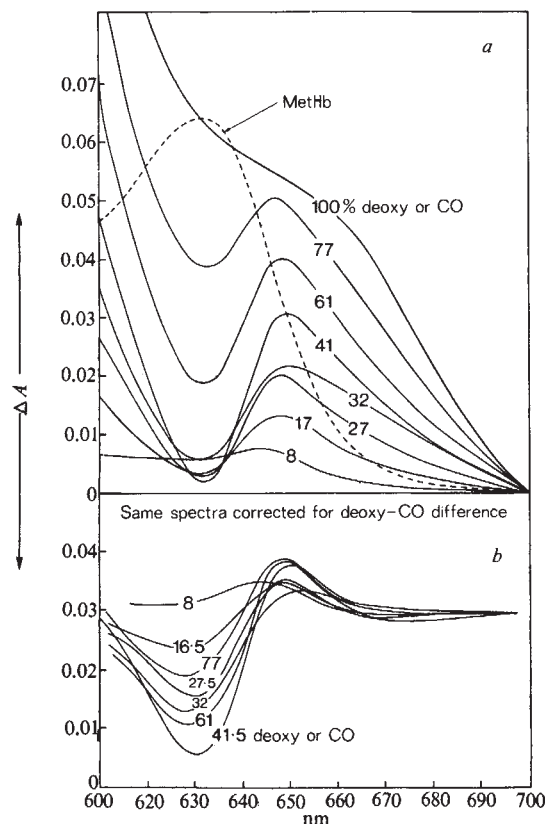


Fig. 1 Difference spectra of 42 μM solutions of deoxy-met minus CO-metHb in 0.05 M *bis* Tris+0.1 M NaCl+200 μM 2,3-DPG at pH 6.5. The numbers indicate the percentage reduction and were determined as follows: one cuvette was filled with pure metHb and another with pure COHb, and in a series of experiments the relative concentrations of these derivatives in the two cuvettes were varied, but so as to keep the total absorbance equivalent to that of a single cuvette filled with a 42 μM haemoglobin solution. The two cuvettes were mounted in tandem and the sums of their spectra were recorded for each of the concentration ratios. From the results a calibration chart was constructed, giving the heights of the deoxy Hb and metHb peaks as a function of these ratios. The spectra of all the original mixtures had been previously recorded with their difference spectra. The percentage COHb and metHb corresponding to these spectra could now be read off the chart. Next, the curve marked "100% deoxy or CO" was reduced in scale corresponding to the percentage actually present in the particular mixture, and subtracted from the curve in *a*, thus giving one of the curves in *b*. Isosbestic points cannot be expected in *a* because four components are present. In *b* they should occur, but are probably obscured by the inaccuracies of the correction procedure outlined and by other errors.

difference spectra rises to a maximum at about 50% reduction and that on dissociation of CO from the hybrids the metHb band at 630 nm is diminished in amplitude and shifted to longer wavelength. The same difference spectra were observed when 2,3-DPG was replaced by IHP. Omission of 2,3-DPG or IHP halved their amplitudes; replacement of *bis* Tris by phosphate buffer made little difference. Fig. 2 shows the same experiment done for the absorption band at 1,000 nm. The corrected difference spectrum (curve A-E) shows a minimum at about 980 nm and a maximum at 1,216 nm, indicative of a reduction in amplitude and shift to longer wavelength on dissociation of CO from the hybrids.

J. V. Kilmarin has made a modified human haemoglobin in which the formation of the C-terminal salt bridges that normally stabilize the deoxy form has been inhibited by the enzymatic removal of arginine 140 α and by blocking the sulphhydryl group of cysteine 93 β with N-ethylmaleimide³. This derivative, N-ethylsuccinimide-DesArg haemoglobin (NES-DesArg Hb), has a high oxygen affinity, lacks all co-operative effects, crystallizes in a form closely related to oxy even when fully deoxygenated⁴, and has an absorption spectrum

like that of the sum of the deoxygenated free α and β subunits, rather than that of native deoxyHb A. These properties show that its allosteric equilibrium is heavily biased towards the *R* form. On combination with CO, deoxymet hybrids of NES-DesArg Hb failed to show any trace of the difference spectra just described. Carboxypeptidase A (CPA)-digested haemoglobin is a derivative from which tyrosines 145 β and histidine 146 β have been removed. It also has a high oxygen affinity, but its human deoxy form does crystallize isomorphously with deoxyHb A (see ref. 4), and it also exhibits a small Bohr effect⁵. These properties indicate that the allosteric constant *L* must be at least 10. Reaction of CPA-digested deoxymet hybrids with CO produced an only just discernible difference spectrum near 630 nm.

Significance of Difference Spectra

According to Smith and Williams a decrease in amplitude and frequency of the metHb absorption bands at 630 and 1,000 nm is associated with an increase in magnetic susceptibility and therefore corresponds to a transition to a state of higher spin⁶. This conclusion has been confirmed by studies of the interaction of methaemoglobin with IHP (unpublished result). There is circumstantial evidence, though as yet no crystallographic proof, that combination of one molecule of IHP with one tetramer of aquo and fluoro-metHb changes their quaternary structure to the *T* form. The reaction of IHP with aquometHb is accompanied by spectral changes which are identical to the ones reported here for the dissociation of CO from deoxymet hybrids. It is also accompanied by an

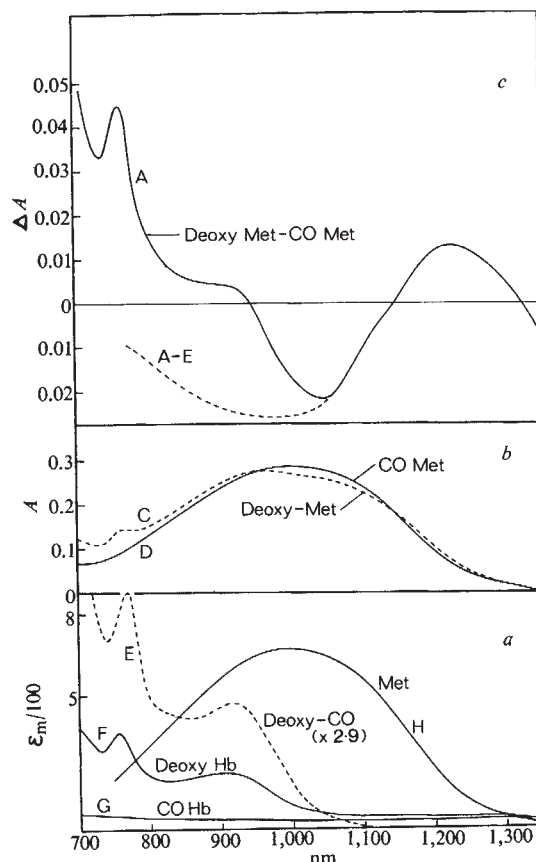
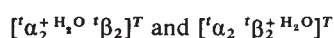


Fig. 2 Near-infrared difference spectra of 160 μM solutions of deoxy-met minus CO-met in 0.05 M *bis* Tris and 0.1 M NaCl and 0.8 M 2,3-DPG at pH 6.5. *a*, Spectra of pure 160 μM solutions of met, deoxy and COHb, together with the difference spectrum deoxy minus COHb $\times 2.9$ enlarged. *b*, Spectra of hybrids after one third of the metHb present in the original solution had been reduced with $\text{Na}_2\text{S}_2\text{O}_4$. *c*, Difference spectrum of the hybrids, A before, A-E after correcting for the deoxy minus CO difference spectrum.

increase in the intensity of the ESR signal at $g=6.0$ and by a marked rise in magnetic susceptibility, which proves that the spectral changes observed in metHb on addition of IHP, and by implication those observed here in the hybrids, do indeed correspond to a transition to higher spin. Electronically this corresponds to a lengthening of the bonds from the iron atom to its octahedral ligands caused by an increased occupancy of the $d_{x^2-y^2}$ and d_{z^2} orbitals which point in the bond directions and a decreased occupancy of the d_{xy} , d_{xz} and d_{yz} orbitals which point between the bond directions^{7,8}. Such a lengthening of the bonds would magnify the displacement of the iron atom and the haem-linked histidine from the plane of the porphyrin ring.

What stereochemical mechanism causes that increase? It was shown that the abnormal ferric subunits of haemoglobin Milwaukee undergo a reversible transition between alternative tertiary structures, one of them isomorphous with deoxy (*t*) and the other with oxy (*r*), without any change of ligand at the ferric irons; the transition is triggered purely by the change in quaternary structure which is brought about by the reversible reaction of the normal ferrous subunits with ligands². This structural transition was accompanied by a spectral change in the metHb absorption band at 620 nm which is the exact equivalent of the one now observed in the band at 630 nm of metHb A. Water and COO^- , as haem ligands, lie close together on the nephelauxetic scale and both metHb derivatives are high spin. It was to be expected therefore that normal deoxy-aquomet hybrids would have the structures



My results, together with those obtained in studying the effect of IHP in the structure of metHb, support this prediction.

It appears that in the iron porphyrins, the electronic structure of the iron arranges itself so that two states, one of higher and the other of lower spin, are very close together in energy. This has long been known for ferric haemoproteins and their derivatives. The energy separation between them for a given ligand depends on the iron-nitrogen bond length (mainly $\text{Fe-N}_{\text{porph}}$ but Fe-N_{His} may also be important) as pictured diagrammatically in Fig. 3*a*. The quaternary structure has two stereochemically different states, one isomorphous with oxy and the other with deoxy. These are also close together in energy and their relative stability depends on the displacement of the iron from the plane of the porphyrin (Fig. 3*b*). Consequently we have a seesaw: changes in quaternary structure alter the tension between the haem-linked histidines and the porphyrin rings, which changes the bond lengths and affects the spin state of the haems, and vice versa.

I now wish to offer a qualitative interpretation of the changes observed in the hyperfine shifted resonances of the ferric haems in haemoglobin M Milwaukee¹². Such shifts are known to arise from contact coupling between the unpaired electrons of the iron and the protons of the porphyrin, or from pseudo-contact interactions with protons of the globin. The magnitude of the shifts of the proton resonances are proportional to $S(S+1)$, where S is the total electronic spin¹³. On this basis a

transition to a state of higher spin, such as is observed in the ferric haems on dissociation of ligand from the ferrous haems, should magnify the hyperfine shifts. Reference to Fig. 2 of Lindstrom *et al.*¹² shows that the magnitudes of the hyperfine shifts are indeed increased in the high spin ferric haem of haemoglobin M Milwaukee, the resonances at -51 and -43 p.p.m. downfield from HDO being shifted further downfield to -54 and -47 p.p.m.

The qualitative agreement between the expected and observed direction of the displacements of the resonances is encouraging and suggests that the changes in the hyperfine shifted lines are attributable directly to the increased spin in the ferric haems, due to the tension which acts on them in the *T* state, though at this stage alternative explanations, based on a redistribution of the spin over the porphyrin ring and on stereochemical changes around the porphyrin ring, cannot be entirely excluded.

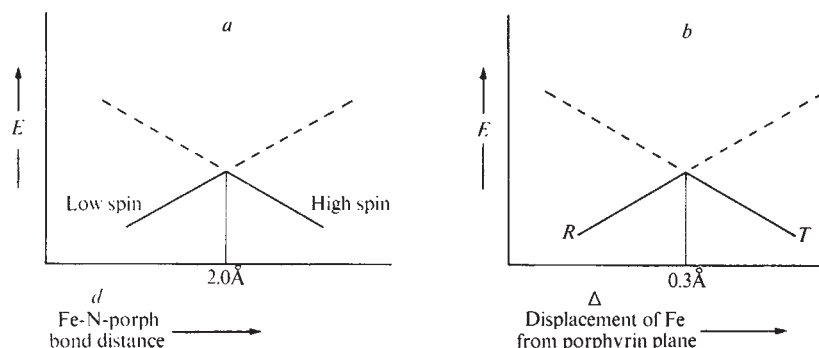
Allosteric Mechanism in Hybrids

The aquomet and Milwaukee hybrids are what might be called pure Monod systems¹⁴, because the reaction of either two subunits with ligand can produce an $R \rightleftharpoons T$ transition which is accompanied by a concerted change in tertiary structure ($r \rightleftharpoons t$) in the other two subunits (Fig. 4). This observation suggests that there are other systems with similar properties: all that is needed is an equilibrium between two tertiary structures so balanced that the formation of a few salt bridges or hydrogen bonds accompanying the $R \rightleftharpoons T$ transition can swing it either way.

Do the hybrids exhibit any interaction in the absence of an $R \rightleftharpoons T$ transition? In the haemoglobins M, the reaction of the ferrous haems with ligand in either pair of normal subunits failed to affect the ferric haems of the abnormal subunits in the absence of a $T \rightarrow R$ transition². The experiments of Shulman and his colleagues clearly demonstrate that in cyanomet hybrids of haemoglobin A the dissociation of ligand from either pair of ferrous subunit fails to affect the haem proton resonances of the ferric subunits unless there is an $R \rightarrow T$ transition^{9,10,11}. At first sight my own failure to find interaction between ferrous and ferric subunit in NES-Des-Arg Hb seemed to confirm this, but then I realized that the answer is ambiguous. Experiments with aquometHb and IHP have shown me that the ferric chains fail to respond to an $R \rightarrow T$ transition if their C-terminal residues have been split off, presumably because the truncated ferric subunits lack the leverage needed to push the penultimate tyrosines into their pockets. The same ambiguity applies to the experiment with CPA-digested haemoglobin.

The crystal structures of the haemoglobins M Milwaukee and Boston have established that the hybrids $\alpha_2\beta_2^+ \text{M}$ and $\alpha_2^+ \beta_2 \text{M}$ both have the *T* structure; they are exactly isomorphous with deoxyhaemoglobin A. In the haemoglobins M, the ferric haems are high spin; the same is true of aquometHb, so that the hybrids $\alpha_2\beta_2^+ \text{H}_2\text{O}$ and $\alpha_2^+ \text{H}_2\text{O} \beta_2$ (in the presence of 2,3-DPG or IHP) must also have the *T* structure. There is no firm evidence yet that this is true for the low spin cyanomet hybrids in the presence of IHP, but I have assumed it to be so

Fig. 3 Changes in free energy as a function of the iron-nitrogen bond distances of (a) the spin state of the haem and (b) the quaternary structure of the haemoglobin molecule. An $R \rightarrow T$ transition lengthens Δ and d , inducing a change to higher spin, and vice versa. The critical distances are approximate.



in the arguments developed here, because the stereochemistry of the $\alpha_1\beta_2$ contact speaks so strongly against intermediate quaternary structures. It should now be possible to test the point by the NMR methods of Lindstrom *et al.* (Fig. 1 of ref. 12). One of the intriguing features of Ogawa and Shulman's results is its demonstration of only two states^{10,11}. Their NMR spectra show side by side the ferric haem proton resonances of the oxy and deoxy states, without any intermediate resonances.

Allosteric Mechanism of Oxygenation

Spectral changes analogous to those now observed on transition of methaemoglobin subunits from the *r* to the *t* state are known to occur on assembly of free deoxy subunits to form tetrameric deoxyhaemoglobin¹⁴. Deoxy derivatives which remain in the *R* state such as NES-DesArg or BME haemoglobin exhibit spectra similar to those of the free deoxy subunits. These observations all point to the conclusion that in deoxy subunits the *R*→*T* transition lengthens the bond distances between the iron and its five neighbours, increases its spin and thereby opposes its combination with oxygen. If this happens to deoxy subunits, then oxy subunits are likely to be affected by the *R*→*T* transition in a similar way, even if there is as yet no experimental evidence to support this.

On this basis both the unliganded and the liganded chains can exist in two alternative states which we may denote as

$$^R\text{deoxy}, ^T\text{deoxy}; ^R\text{oxy} \text{ and } ^T\text{oxy}$$

R and *T* referring to their conformations in the quaternary *R* and *T* structures respectively.

How are these states related stereochemically? X-ray analysis has shown large stereochemical differences between ^{*R*}oxy and ^{*R*}deoxy (see ref. 1), and between ^{*T*}deoxy and ^{*T*}oxy (see ref. 16). These have convinced me that the major changes in tertiary structure are sequential; that each chain undergoes a marked stereochemical change as it combines with or releases oxygen, even if the quaternary structure is held rigid. Of the stereochemical differences between ^{*R*}deoxy and ^{*T*}deoxy, or ^{*R*}oxy and ^{*T*}oxy, we know nothing as yet.

Even though X-ray studies show the change in tertiary structure to be sequential with oxygen uptake, no one has produced convincing evidence that the oxygenation of one subunit significantly affects the oxygen affinity of the others

unless that reaction is accompanied by a change of quaternary structure. In this sense, and because both the oxy and the deoxy subunits can apparently exist in two alternative states, one constrained and the other relaxed, the mechanism resembles the concerted one of Monod *et al.*¹⁴ rather than the sequential one of Koshland *et al.*¹⁶. In fact it should be possible to describe the reaction of haemoglobin with oxygen in terms of four equilibrium constants ^{*T*}*K*_α, ^{*R*}*K*_β, ^{*R*}*K*_α and ^{*R*}*K*_β, and the corresponding eight kinetic constants, together with the allosteric constant *L*.

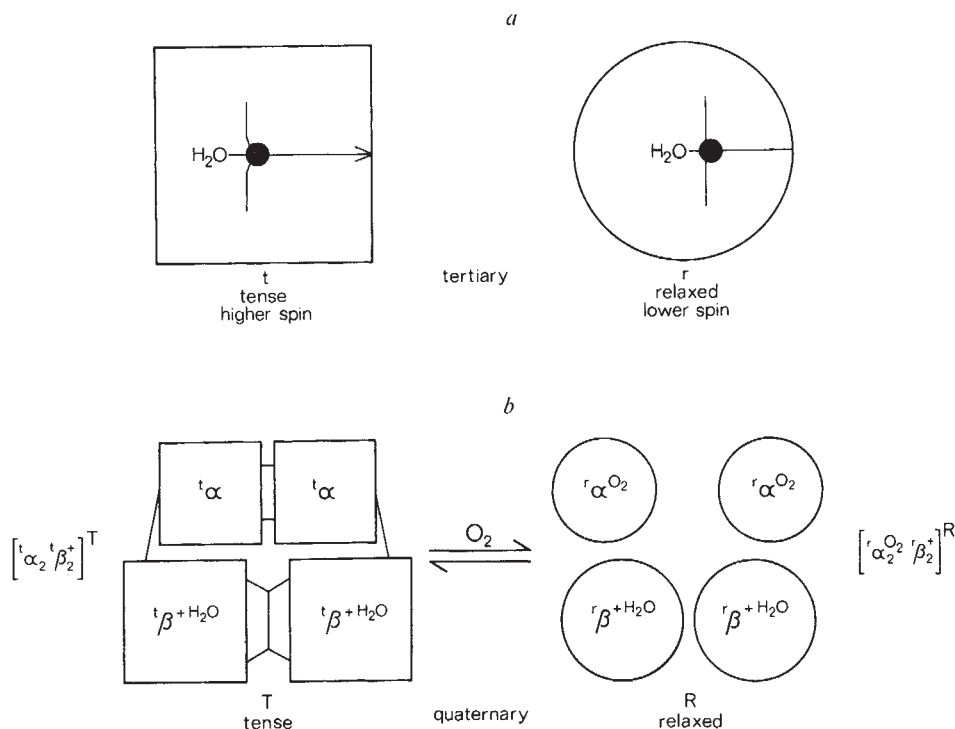
We may now begin to understand the variations in oxygen affinity, other than those due simply to changes in allosteric equilibrium, which characterize different myoglobins and haemoglobins. There should be a simple inverse relationship between intrinsic oxygen affinity, on the one hand, and electronic spin of the ferrous deoxy haems on the other. The few available data are consistent with this idea¹⁷. In deoxyHb a resonance line assigned to methyl protons of the β haems appears downfield at -17.9 p.p.m. In all but one of several haemoglobins with high oxygen affinity that line is displaced upfield. (The exception is spin-labelled haemoglobin where the increased total electronic spin shifts lines further downfield.) In CPA-digested deoxyHb (Des Tyr 145→His 146) and in deoxyHbs Zürich (His 63β→Arg) and Kempsey (Asp 99β→Asn) upfield displacements of between 1.8 and 2.6 p.p.m. are recorded. In haemoglobin Zürich the β haems are actually known to be low spin¹⁸; the NMR data suggest that the same may also be true for the other two haemoglobins. The upfield displacements of the line in deoxyHb Chesapeake (Arg 92α→Leu), Capetown (Arg 92α→Gln) and Yakima (Asp 99β→His) are ten–twenty times smaller and may be due to stereochemical factors rather than spin transitions. Besides, their high oxygen affinities are probably caused by shifts of the allosteric equilibria towards the *R* structure rather than high affinity of the subunits within the *T* structure.

Principal Conclusions

(1) In mixed state haemoglobins dissociation of ligand from the ferrous subunits causes a change from the normal quaternary oxy to the normal deoxy structure.

(2) That change causes the tertiary structure of the ferric subunits to alter; the resulting tension at the haems causes an electronic transition which lengthens the bond distances

Fig. 4 *a*, Diagrammatic sketch showing the relaxation of tension at the haem on going from the tertiary deoxy to the oxy structure. *b*, Concerted change of tertiary and quaternary structure on binding oxygen or CO to the ferrous subunits. The clamp between the β subunits in the *T* structure represents 2,3-DPG; the other links represent the salt bridges.



between the iron atom and its six neighbours, and raises the spin.

(3) Ferrous subunits also change to a constrained state of higher spin on transition to the quaternary deoxy structure. I suggest that this higher spin and the associated increases in bond lengths are the principal causes of the low oxygen affinity of the quaternary deoxy structure.

(4) On this basis haem-haem interaction consists of a change in tension at the haem, brought about by a transition between two alternative quaternary structures. In the quaternary oxy form the haem structure is relaxed, making the oxygen affinity high; in the quaternary deoxy form it is constrained by the contacts between the protein subunits, making the oxygen affinity low. Combination of ligand with, or dissociation of ligand from, one pair of haems has no significant effect on the spin state at the other pair unless that reaction is accompanied by a change of quaternary structure, at least not in methaemoglobin hybrids. There is evidence that the constraints at the haems are much weakened, even in the normal quaternary deoxy structure, if the penultimate tyrosines (HS2) are either removed or replaced by other residues, or if the C-terminal salt bridges are broken.

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Synthetic RNA-dependent DNA Polymerase Activity in Normal Rat Liver and Hepatomas

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Normal adult rat liver contains a high level of a synthetic RNA-dependent DNA polymerase activity, which is distinct from cellular DNA-dependent polymerases. It uses poly(dT)·poly(rA) and poly(rA)·poly(rU) as templates but has little or no response to DNA or several single-stranded RNAs.

THE RNA-dependent DNA polymerases (reverse transcriptases) found in various oncogenic RNA viruses¹⁻³ give much more polymerization with synthetic polynucleoside tem-

plates^{4,10,11} than with viral RNA. Enzymes which respond to synthetic templates have been found in other RNA viruses^{5,6}, in normal phytohaemagglutinin-stimulated lymphocytes⁷ and in cultured non-malignant fibroblasts⁸. Although both normal and virus-transformed cells contain the latter enzyme activity, Ross *et al.*⁹ have shown that normal BALB/3T3 cells contain two DNA polymerases and MSV-transformed BALB/3T3 cells contain an additional enzyme which has properties of the polymerase isolated from murine leukaemia virus. The observation that the murine cellular enzyme is physically and antigenically distinct from the viral enzyme makes it essential to purify and characterize these polymerases from other malignant and non-malignant cells. Here we report that normal adult rat liver possesses a high level of synthetic RNA-dependent DNA polymerase, providing a convenient source for the large scale preparation of a cellular type enzyme.

We have used principally synthetic double-stranded RNAs and synthetic DNA-RNA hybrid templates which have proven useful for detecting RNA-dependent DNA polymerase activities in RNA tumour viruses^{10,11}. In this article we define RNA-dependent DNA polymerase ("RD-DP") as an enzyme

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which preferentially responds to either poly(rA)·poly(rU) or poly(dT)·poly(rA) and polymerizes ^3H -TMP into an acid-insoluble, RNAase-resistant, material. DNA-dependent DNA polymerases ("DD-DPs") refer to enzymes which respond to both natural (calf thymus) and synthetic [poly d(A-T)] DNAs but have little or no activity with synthetic DNA-RNA hybrids or double stranded RNAs. Although DNA polymerases from *Escherichia coli* and calf thymus can use poly(dT)·poly(rA) and poly(rA)·poly(rU) as templates^{4,18-20}, the DNA-dependent DNA polymerase(s) of rat liver cannot use these templates. In addition, the RNA-dependent DNA polymerase activity which we purified does not accept natural or synthetic DNA as templates. It is not clear, however, that the liver RD-DP is a true reverse transcriptase, as defined by Goodman and Spiegelman⁴.

Fractionation of Liver

The components of the assay system and the method of processing samples for radioactivity measurements are given in Table 1. Mature Sprague-Dawley rats (100-150 g) were decapitated and their livers were excised and placed in ice cold buffer A (0.1 M Tris-HCl buffer, pH 8.3; 0.01 M MgCl_2 , 0.005 M 2-mercaptoethanol). All subsequent fractionation steps were performed at 4° C. After rinsing several times with buffer, livers were resuspended in buffer A (30 ml./10 g liver), minced using scissors and homogenized for 30 s at the lowest speed of a 'Silverson' homogenizer. This procedure, as judged by phase-contrast microscopy, ruptures the cells but leaves most nuclei intact. The homogenate was centrifuged for 15 min at 2,000g in the 8 × 50 rotor of an 'MSE model 18' centrifuge. The supernatant was carefully decanted and the pellet suspended in buffer A (30 ml./original 10 g liver) and recentrifuged at 2,000g for 15 min. The second supernatant was pooled with the first and the pellet resuspended by homogenization in buffer A. The pooled supernatants contained 60-80% of the total DD-DP activity, while the pellet contained 75-85% of the total RD-DP activity when assayed with poly(rA)·poly(rU) or poly(dT)·poly(rA).

Table 1 Purification Scheme for RNA-dependent DNA Polymerase from 10 g (Wet Weight) of Rat Liver

Step	Enzyme fraction	Total protein mg	Total dTrA units*	Specific activity units/mg	% yield
-	Homogenate	2,095	75,800	36	—
1	2,000g pellet	1,252	68,000†	54	90
2	30,000g 'Nonidet' extract	550	52,000	94	69
3	100,000g supernatant	480	50,000	104	66
4	30-45% AS	170	36,500	215	48
5	G-100	64	29,500	460	39
6	DEAE cellulose	11	15,000	1,360	20

* 1 unit = the polymerization of 1 pmol of ^3H -TMP in a 20 min reaction. The values given for the homogenate and 2,000g pellet are those obtained after treatment (4° C, 30 min) with 'Nonidet P40' (1% final concentration). The standard assay (0.1 ml.) contained: Tris-HCl buffer, pH 8.3, 0.03 M; MgCl_2 , 0.01 M; KCl, 0.04 M; dithiothreitol, 0.001 M; dATP, 1.6×10^{-4} M; dTTP (1.6×10^{-4} M, specific activity, 400-550 c.p.m./pmol) and one of the following templates: poly(rA)·poly(rU), 5 µg; poly(dT)·poly(rA), 0.5 µg; poly d(A-T), 5 µg. Reactions which contained natural DNA or RNA templates had in addition 1.6×10^{-4} M dGTP and dCTP. Assays were incubated at 37° C for 20 min, unless specified otherwise, and then stopped by the addition of 5 ml. TCA-phosphate solution (see below). Acid precipitable material was collected on glass fibre filters ('Whatman GF/A') and washed with the following mixture: 100% TCA : saturated trisodium orthophosphate : tetrasodium pyrophosphate : water (1 : 1 : 1 : 7). The samples were counted in a liquid scintillation counter.

† The combined 2,000g supernatants contained 25,000 poly(dT)·poly(rA) units. This activity, when subjected to steps 3-6, fractionated in the same manner as the activity extracted from the 2,000g pellet.

Centrifugation of the 2,000g supernatant fraction at 10,000g to yield a mitochondrial fraction and then at 100,000g to yield a microsomal fraction revealed that negligible amounts of DD-DP and RD-DP activity sedimented with either of these two cell fractions. The fact that most of the RD-DP activity was found in the low speed pellet suggests that this enzyme is present in the nucleus or is bound to large membrane fragments (possibly derived from the plasma membrane). Attempts to demonstrate RD-DP activity in highly purified preparations of liver nuclei have given variable results.

The amount of either DNA or RNA-dependent polymerase activity detected in the 2,000g pellet was increased three-five-fold by the presence of 1% 'Nonidet P40'. 'Nonidet' did not, however, increase the activity of either RD-DP or DD-DP in the supernatant fraction. The RD-DP in the 2,000g pellet can be solubilized with detergents, by repeated homogenization in EDTA-containing buffers, by freezing and thawing, or by leaching at 4° C for 1 or 2 days. To solubilize the enzyme, the 2,000g pellet was suspended in buffer A and 'Nonidet' was added to a final concentration of 1%. After standing for 30 min at 4° C the material was centrifuged for 30 min at 30,000g. The resulting pellet was re-extracted with 1% 'Nonidet' in buffer A and the centrifugation repeated. The two 30,000g supernatant fractions were combined and centrifuged at 100,000g for 2 h in the SW27 rotor of a 'Beckman model L265B' ultracentrifuge. The 100,000g supernatant contained 60-80% of the RD-DP activity present in the original 2,000g pellet.

The 100,000g supernatant fraction (100 ml.) was adjusted to 30% saturation with ammonium sulphate by adding slowly with continuous mixing, 58 ml. of an ammonium sulphate solution containing 47.5 g/100 ml. (pH 7.0). After 20 min this was centrifuged at 30,000g for 20 min. The clear supernatant layer was removed and a viscous yellow layer and a pellet fraction (both containing large amounts of 'Nonidet') were discarded. This step removed most of the detergent which otherwise interfered with subsequent fractionation steps. It did result, however, in some loss (0-25%) of the RD-DP activity (Table 2). Solid ammonium sulphate was added to the above supernatant fraction to a final concentration of 45% (an additional 9.3 g/100 ml.) and after 20 min the precipitate was sedimented at 10,000g for 20 min. This pellet contained most of the RD-DP activity. Most of the DD-DP activity was in the supernatant fraction from which it was precipitated by raising the ammonium sulphate concentration to 60% saturation (an additional 10 g/100 ml.). The latter two pellets were dissolved in one-fifth their original volume of buffer B (0.02 M Tris-HCl buffer, pH 8.0; 0.04 M KCl, 0.001 M 2-mercaptoethanol and 0.001 M EDTA).

Table 2 Separation of RNA-dependent and DNA-dependent DNA Polymerases by Ammonium Sulphate Fractionation

Ammonium sulphate fraction	Units of enzyme activity		
	Poly dTrA	Poly rArU	Poly d(A-T)
0-30	2,900	1,600	1,100
30-45	21,600	6,400	8,500
45-60	None detected	None detected	66,000

The sample subjected to fractionation (step 3, Table 1) contained the following units of enzyme: poly dTrA, 32,000; poly rArU, 10,300; poly d(A-T), 82,400. The percentage total recovery was: poly dTrA, 76%; poly rArU, 78%; poly d(A-T), 92%.

The ammonium sulphate fractionation gave appreciable separation of the two types of polymerase activities (Table 2). The 30-45% fraction contained 80-95% of the RD-DP activity and 5-15% of the DD-DP activity; whereas the 45-60% fraction contained 85-95% of the DD-DP activity but none of the RD-DP.

The RD-DP activity in the 30-45% ammonium sulphate

fraction was further purified by 'Sephadex G-100' column chromatography (Fig. 1). It eluted just after the void volume and in advance of the residual DD-DP activity. The separate elution profiles of the two enzyme activities provide further evidence that they are distinct entities. The DD-DP activity eluted as two distinct peaks, although the ratios of activities in these peaks varied somewhat from preparation to preparation. Weissbach *et al.*¹² reported that HeLa cells also contain two separable DNA polymerases. The elution profiles of the RD-DP activity were identical whether assayed with poly(dT)·poly(rA) or poly(rA)·poly(rU). The activity with these two templates also co-chromatographed on 'Sephadex G-200'. Although in most preparations only a single peak of RD-DP activity was observed, on occasion a second minor peak was found. Fractionation of these preparations by isoelectric focusing (Fig. 2) also indicated that the RD-DP may be further resolved into a major and minor component.

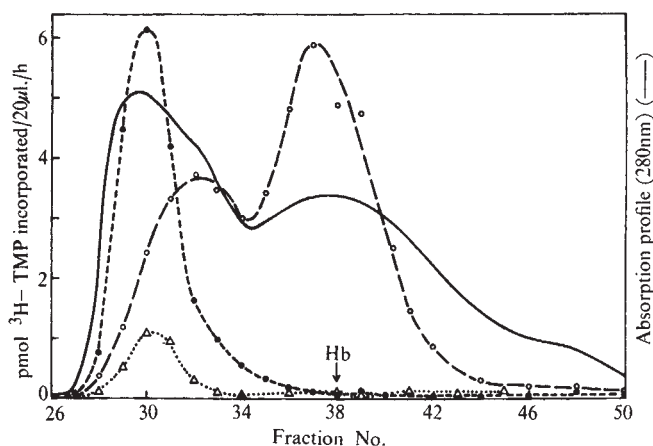


Fig. 1 Chromatography of rat liver DNA polymerases on 'Sephadex G-100'. A 1.5 ml. sample of a 30-45% ammonium sulphate fraction was applied to a 200 ml. column of G-100 (2×65 cm) which was equilibrated and washed with buffer B. 2.0 fractions were collected at a flow rate of 6 ml./h. A 20 µl. aliquot of each indicated fraction was incubated for 60 min with each of three synthetic templates in standard 0.1 ml. reactions (see Table 1): poly d(A-T) (○); poly dTrA (●); poly rArU (△). Hb denotes the peak tube of a rat haemoglobin marker.

The major fractions active with poly(dT)·poly(rA) and poly(rA)·poly(rU) obtained from the G-100 column were pooled and further fractionated on a DEAE cellulose column by stepwise elution. A single peak of activity again responding to both of these templates eluted with 0.24 M KCl. This was completely resolved from a small peak of activity which eluted at 0.34 M KCl and responded to poly d(A-T). The latter was completely inactive with poly(dT)·poly(rA) or poly(rA)·poly(rU). The RD-DP enzyme fraction obtained from the DEAE column had approximately forty-fifty times the specific activity of the crude homogenate, with a yield of approximately 20% (Table 1).

Studies in progress suggest that a greater purity and yield of RD-DP can be achieved by the following procedure. Homogenization is carried out in buffer B and the homogenate is left at 4° C for 1-2 days to "leach-out" RD-DP activity. The homogenate is then centrifuged at 100,000g for 2 h and the supernatant fraction made 0.1% in protamine sulphate. After stirring for 30 min, the precipitate is removed by centrifugation and the soluble fraction adjusted to 60% ammonium sulphate saturation. This solution is stirred for 20 min, the precipitate collected by centrifugation, dissolved in buffer B, dialysed overnight, and applied slowly (5 ml./h) to a column of DNA-cellulose²³. The column is washed with buffer B until all non-absorbed protein is removed. The RD-DP and DD-DP activities are then eluted with buffer B containing 0.6 M KCl, vacuum dialysed overnight in buffer B, and applied to a

G-100 column, as described above. The RD-DP activity from the G-100 column is 200 times purified and yields 40% of that in crude extracts.

Properties of Liver RD-DP

A partially purified preparation of RD-DP gave a linear time course for at least 2 h (Fig. 3). The enzyme from the 30-45% ammonium sulphate step gave linear response over the range 5 to 80 µg of protein/0.1 ml. assay system. Although the enzyme was usually assayed with 10 mM Mg²⁺, Mn²⁺ gave three times greater activity. The Mg²⁺ optimum occurred over a wide range (5-20 mM) whereas Mn²⁺ gave a sharp maximum at 0.5 mM. The RD-DP activity of tumour viruses is also maximal with Mn²⁺ (ref. 11). Despite the greater effectiveness of Mn²⁺ we prefer to use Mg²⁺ since it gives more reproducible results.

With poly(dT)·(rA) as template, enzyme activity rose steeply as the pH was increased from 6.0 to 8.0, plateaued from pH 8.0 to 9.5, and decreased above pH 9.5. Increasing KCl (0.05 to 0.4 M) resulted in progressive inhibition of the RD-DP activity (50% inhibition at 0.2 M KCl). Therefore enzyme activity in column fractions containing a high concentration of salt may be underestimated.

During the fractionation of rat liver, the poly(rA)·poly(rU) activity paralleled the poly(dT)·poly(rA) activity. Heat inactivation of a partially purified preparation of RD-DP indicated that at 50° C both activities decayed in parallel with a *T*_{0.5} of approximately 30 min. On the other hand, with storage at 4° C in buffer B, the poly(dT)·poly(rA) activity was extremely stable but the poly(rA)·poly(rU) activity gradually decayed. The latter activity was also much more labile in column fractions. Freezing, even in the presence of reducing agents, glycerol or ethylene-glycol, resulted in complete inactivation of both activities. The greater lability of the poly(rA)·poly(rU) activity might be due to loss of a cofactor, an active site, or a subunit specific for double stranded RNA. Alternatively there may be separate but as yet unresolved enzymes which respond to the two types of templates.

The template specificity of the RD-DP enzyme is given in Table 4. Although the data presented were obtained with a

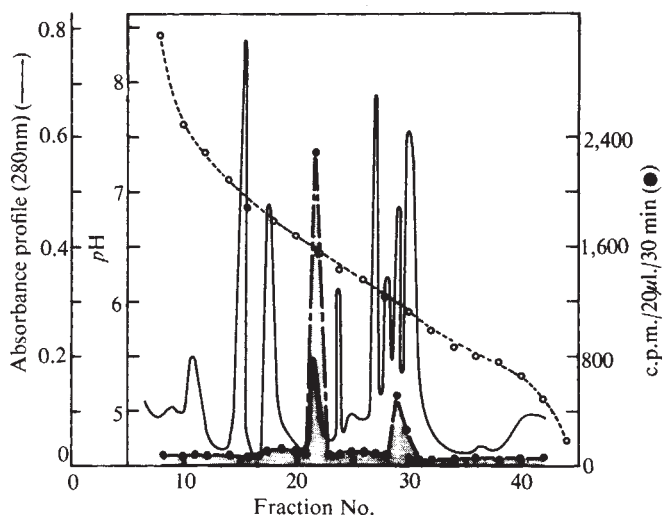


Fig. 2 Fractionation of rat liver RD-DP activity by isoelectric focusing. The 110 ml. column contained a 2% solution of ampholines (pH 5-8) in a standard sucrose gradient and a 1.0 ml. sample of enzyme (step 3 purified—Table 1). After focusing for 48 h at 500 V fractions of 2.0 ml. were collected, their pHs determined, and then neutralized by the addition of 0.2 ml. of 1.0 M Tris-HCl buffer, pH 8.3. 20 µl. aliquots of the indicated fractions were assayed with poly dTrA and poly d(A-T) as templates. The total recovery of RD-DP activity, as measured by the poly dTrA directed reactions, was greater than 85%. No poly d(A-T) stimulated activity was recovered after focusing, although the sample applied contained DD-DP.

Table 3 Units* of Enzyme Activity per Gram of Tissue (Wet Weight)

Template	Units of enzyme activity $\times 10^{-3}$	
	Normal liver †	Hepatoma ‡
Poly dTrA	9.3 (7.6–10.5)	17.0 (13.1–22.3)
Poly rArU	1.1 (0.9–1.3)	2.5 (2.1–3.4)
Poly d(A–T)	28.4 (21.5–39.0)	27.5 (21.0–36.5)

* The numbers expressed are the sum of the activities in the 2,000g supernatant and pellet fractions after treatment with 'Nonidet P40' (see Table 1).

† Average of five different preparations (range).

‡ Average of four different preparations (range).

'Nonidet' solubilized enzyme fraction from a hepatoma (see below) similar results were obtained with the corresponding fraction from rat liver. Of the polymers tested thus far, poly(dT)·poly(rA) was the best template; poly(rA)·poly(rU) was approximately 15% as effective. The double stranded alternating copolymers, poly d(A–T) and poly r(A–U) were inactive as was native or denatured calf thymus DNA, either before or after "nicking" with DNAase. The following naturally occurring or synthetic "single stranded" RNAs were also inactive as templates: tRNA, ribosomal RNA, poly(rA) and poly(rU).

Table 4 Template Specificity of RNA-dependent DNA Polymerase

Template*	Template concentration/ assay μg	C.p.m.	Pmol radio-active TMP polymerized
None	—	92	—
Poly dTrA	0.5	4,950	12.3
Poly rArU	7.5	826	1.9
tRNA	5.0	104	<0.05
rRNA	10	106	<0.05
Poly r(A–U)	7.5	113	<0.05
Poly r(A, U)	20	108	<0.05
Poly rA	5.0	90	<0.05
Poly rU	5.0	97	<0.05
Poly d(A–T) †	5.0	115	<0.05
Native calf thymus DNA †	6.0	89	<0.05
Denatured calf thymus DNA †	6.0	96	<0.05

Standard 0.1 ml. reactions incubated for 40 min with 20 μg of hepatoma protein (step 3 purified, specific activity 300 poly dTrA units/mg protein). Specific activity of radioactive deoxynucleoside triphosphates was approximately 400 c.p.m./pmol.

* All templates were tested at three different concentrations. The values given are the highest concentrations used.

† Since the enzyme fraction described above still contained a small amount of DNA-dependent DNA polymerase activity the response to DNA templates was determined with a step 6 enzyme fraction (specific activity 1,275 dTrA units/mg protein). dTrA control = 5,800 c.p.m.; no template = 86 c.p.m. The template specificity of step 6 purified enzyme was qualitatively identical to that of the step 3 enzyme shown above.

The unusual template specificity of liver RD–DP does not appear to be due to contamination with nucleases. When RD–DP (steps 4, 5 or 6 purified) was incubated for 20 min with ^3H -poly d(A–T), ^3H -poly A or ^3H -ribosomal RNA, at identical template concentrations as used in standard assays, less than 15% of the counts were rendered acid soluble. In addition, the RD–DP fractions did not inhibit DD–DP activity directed by either calf thymus DNA or poly d(A–T). Conversely, the DD–DP enzymes in the 45–60% ammonium sulphate cut (or later stages of purification) did not appreciably degrade ^3H -poly(rA)·poly(rU) or ^3H -poly(dT)·poly(rA) nor did they inhibit the RD–DP activity.

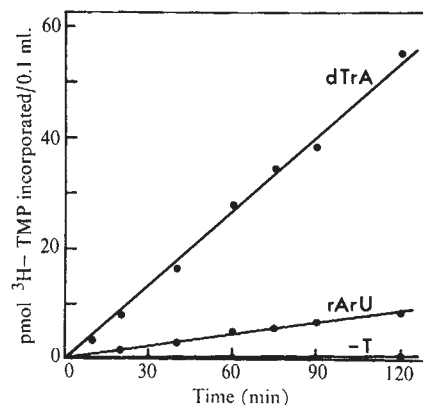


Fig. 3 Time course of rat liver RD–DP activity with the templates poly dTrA and poly rArU. 100 μl . aliquots were withdrawn at the indicated times from 0.8 ml. reactions and acid precipitated. The reactions contained 375 μg of step 4 purified protein (specific activity 180 poly dTrA units/mg). Specific activity of ^3H -TTP was 515 c.p.m./pmol.

Studies with Hepatomas

Since RNA tumour viruses and MSV-transformed BALB/3T3 cells contain a different RD–DP activity to non-transformed cells⁹, it was of interest to determine whether any quantitative or qualitative differences could be found between the RD–DP activity of normal liver and similar activities in liver tumours induced by a chemical carcinogen. These studies employed either primary tumours¹³ from rats fed dimethylaminoazobenzene (DAB) or a hepatoma induced with DAB and carried by serial transplantation. Similar results were obtained with the two sources of material. The fractionation procedure was similar to that described above for normal rat liver.

Per gram wet weight of tissue, crude homogenates of hepatomas had approximately twice the RD–DP activity of normal rat liver, when assayed with either poly(dT)·poly(rA) or poly(rA)·poly(rU), (Table 3). On the other hand, crude homogenates of hepatoma and liver had equal amounts of DD–DP activity when assayed with poly d(A–T). During the initial stage of fractionation of hepatomas a greater percentage of the RD–DP activity was soluble than with rat liver. With hepatomas 50–70% of the RD–DP activity was in the 2,000g supernatant fraction, and was active in the absence of detergents; only 30–50% was in the 2,000g pellet. (The corresponding figures for rat liver were 15–25% and 75–85% respectively.) Despite these differences, the behaviour of hepatoma RD–DP and DD–DP, during subsequent ammonium sulphate and column fractionation, was the same as that obtained with the corresponding activities from rat liver. This was true with the enzymes obtained directly from the 2,000g supernatant fraction as well as those solubilized from the 2,000g pellet fraction with 1% 'Nonidet'.

Our results demonstrate that the liver of adult rats contains a DNA polymerase "RD–DP" which responds to synthetic double stranded DNA–RNA and RNA–RNA hybrids but not to natural or synthetic DNA. A similar polymerase has been described in phytohaemagglutinin stimulated lymphocytes⁷. The ready availability of rat liver, as well as previous extensive biochemical characterization of this tissue, makes it a convenient source for further purification and characterization of this enzyme.

The biological significance of this enzyme is not known. Since we used synthetic templates, it is not clear that this enzyme is a true reverse transcriptase. It is unlikely that liver RD–DP is involved in conventional DNA replication *in vivo*, since while demonstrating a high activity with synthetic DNA–RNA and RNA–RNA hybrids, it has, in contrast to DNA polymerase I of *E. coli* and calf thymus DNA polymerase^{18–20}, negligible activity with single or double stranded DNA templates. We cannot, however, exclude the possibility that the unusual template specificity of this enzyme represents an

in vitro artefact or that the enzyme be involved in the *in vivo* repair or degradation of nucleic acid. Studies are in progress to determine whether viral RNAs and naturally occurring single stranded RNAs complexed with oligo(dT)^{21,22} serve as templates for the liver enzyme.

Temin has postulated in his "protovirus theory", a possible role of reverse transcriptase activity during normal development and differentiation¹⁴, and others have suggested a role of reverse transcriptase in gene amplification¹⁵. In view of these possibilities, a search for a double stranded RNA or DNA-RNA hybrid in normal rat liver which might serve as a template for liver RD-DP is warranted. Harel and Montagnier¹⁶ have shown that rat liver contains a double stranded RNA which hybridizes specifically with rat liver DNA.

The presence of multiple types of DNA polymerases in normal liver and in hepatomas makes this system an extremely attractive one for comparing events occurring during chemical carcinogenesis with those accompanying transformation with RNA tumour viruses. Ross *et al.*⁹ have presented evidence that in extracts of a 3T3 cell line transformed by MSV one can demonstrate a composite of both the normal cell and the sarcoma virus-associated polymerase activities. If chemical carcinogens transform cells by activating latent RNA tumour viruses or "oncogenes"¹⁷, then similar differences might be observed in a comparison between the polymerase profiles of normal liver and chemically induced hepatomas. In our preliminary studies, no major qualitative differences between the synthetic RNA-dependent DNA polymerases of these two tissues were found. More extensive purification of the normal and hepatoma polymerases is required, however, to obtain a definitive answer to this question.

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Evidence for Solar Particle Production above ~ 75 GeV

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Observations of solar particles in the energy region above ~ 75 GeV provide good evidence for particle acceleration during the initial phase of a solar flare.

We present here the first direct experimental evidence for solar particle production in the energy region above the maximum vertical geomagnetic cutoff. This is the outcome of an experiment specifically designed for the detection of solar generated particles in the energy region above

~ 75 GeV, full details of which have been presented elsewhere¹.

So far, there has been essentially no experimental work on solar particle production above 75 GeV, chiefly because the upper limiting solar production rigidity was believed to be considerably below this region (see ref. 2).

Our system was designed to look for a highly anisotropic particle flux, exhibiting a preferential arrival direction in a coordinate system fixed with respect to the Earth-Sun position. The direction of interest corresponds to that of the mean, large-scale interplanetary magnetic field in the vicinity of the Earth; namely $\sim 45^\circ$ west of the Earth-Sun line, and essentially in the plane of the ecliptic.

Our detector is a narrow-angle muon telescope, consist-

ing of three in-line plastic scintillators and two narrow-gap spark chambers. Each scintillator is 30.5 cm in diameter and 5.1 cm thick. The detector is 2.7 m long, with the first and third scintillators defining the telescope geometry. The resulting geometric factor is $\sim 7 \text{ cm}^2 \text{ sr}$, with a corresponding opening angle of $\sim 12.6^\circ$. To achieve the desired orientation, the detector is mounted on a solar tracking equatorial mount.

For specific resolution of a very high energy solar particle component, the detecting system is located underground (vertical depth 200 m water equivalent) in the Colorado School of Mines Experimental Mine, Idaho Springs, Colorado (geographic longitude: 105.6° W ; geographic latitude: $+39.7^\circ$; elevation: 2,500 m above sea level).

The system has been in operation for $\sim 3,000 \text{ h}$. Unfortunately, we were not operating during the two large-scale ground level events which occurred during 1971. But on October 3, 1971, a positive correlation was obtained between a significant intensity increase at our detector and a specific solar event. This was the first statistically signifi-

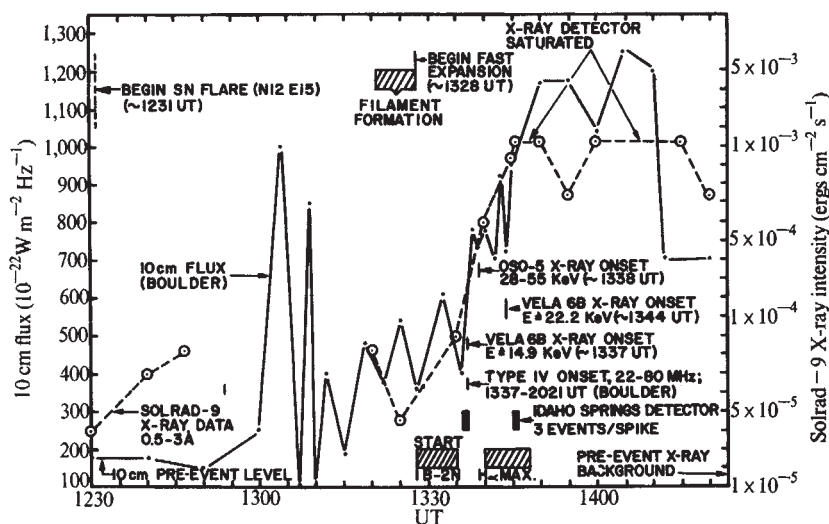
val about the event period, routine operational checks had been performed. In all cases, the system was found to be operating satisfactorily. We feel strongly that the detected event was real, and did not arise from a system malfunction.

The rock overburden viewed by the detector during the event was fairly uniform over the entire opening angle of the instrument. Using the technique of Ahluwalia³, the effective primary threshold energy was calculated as $100 \pm 25 \text{ GeV}$.

The correlated solar event originated from Boulder Sunspot Group 280 (McMath Plage Region 11537) located at $\sim \text{N}13 \text{ E}13$. No other region exhibited any energetic activity for a period of days about the event time. The event was classified as an M8, Importance 1B-2N flare which began at $\sim 1328 \text{ UT}$ on October 3.

Fig. 1 shows a portion of the preliminary data relating to the electromagnetic characteristics of the flare, and the corresponding association with the particle event as recorded by our detector. Most of the flare-related data are as

Fig. 1 Electromagnetic characteristics of the October 3, 1971, flare. Data on the filament structure were obtained from a scan of the Canary Islands H_α patrol films. The Solrad-9 data-break, associated with the 10 cm pre-flare burst, results from an eclipsing of the satellite by the Earth. No X-ray data above 55 keV are yet available.



cant increase, independent of solar conditions, recorded by our detector.

The event consisted of two particle groups, each group containing at least three muons in a 1 min interval. The two groups were separated by 9 min. Specific group times were 1336 and 1345 UT, accurate to $\pm 3 \text{ s}$. It was possible to ascertain that each of the three counts (muons) per group was separated in time by at least 200 ms. By fixing the detector position, the background rate at the event orientation was determined to be $\sim 1.7 \text{ counts h}^{-1}$ —in agreement with calculated rates. Thus the event rate was approximately twenty times background during the period of interest.

A χ^2 analysis of the fixed detector data indicated that the parent distribution was Poisson in nature as expected. Applying Poisson statistics to our results, the chance probability of obtaining any one of the two particle groups is ~ 3 parts in 10^6 . The chance probability of both groups occurring in one 10 min interval is approximately a factor of 10 less.

A complete system check had been made 39 h before the event time. Approximately 34 h after the event occurred, a second complete check was made. In the inter-

yet unpublished, and were obtained through private communication with the various investigators involved. The time spread that appears for both the flare start and H_α maximum results from various observing stations reporting different times.

The flare itself exhibited a twin-filament structure forming along the neutral line of the sunspot group—typical of most flares producing ground level events and strong PCA (ref. 4). Importance 2 and 3 SID were associated with the flare. No data are yet available on the instantaneous magnetic configuration of the group, but Mount Wilson Observatory classified the group as $\beta\text{P}5$ approximately 21 h before the flare, and again approximately 5 h after the event.

Preliminary data on the associated satellite proton event have been obtained for the ATS-1 satellite and Pioneer-6 space probe (private communications). The ATS-1 21-70 MeV channel indicated a particle enhancement starting at about 1430 UT on October 3; the 5-21 MeV onset occurred at about 1700 UT. Peak flux in both channels was obtained at $\sim 1100 \text{ UT}$ on October 4. The Pioneer-6 data are considerably less complete at this time, with no data available for October 3. But on October 4 and 5,

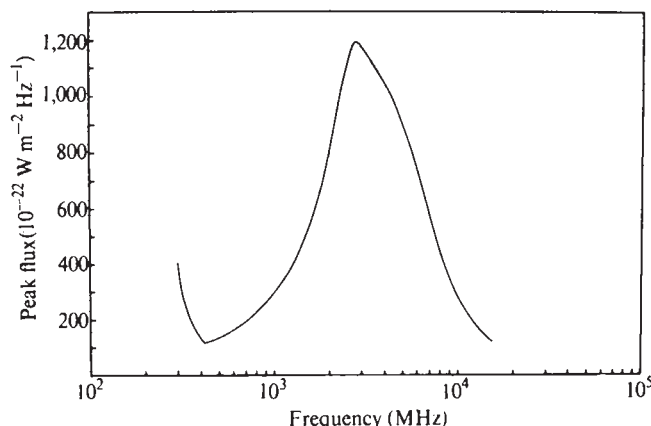


Fig. 2 Flare associated radio spectrum. Data obtained from the Boulder and Sagamore Hill Radio Observatories (private communications).

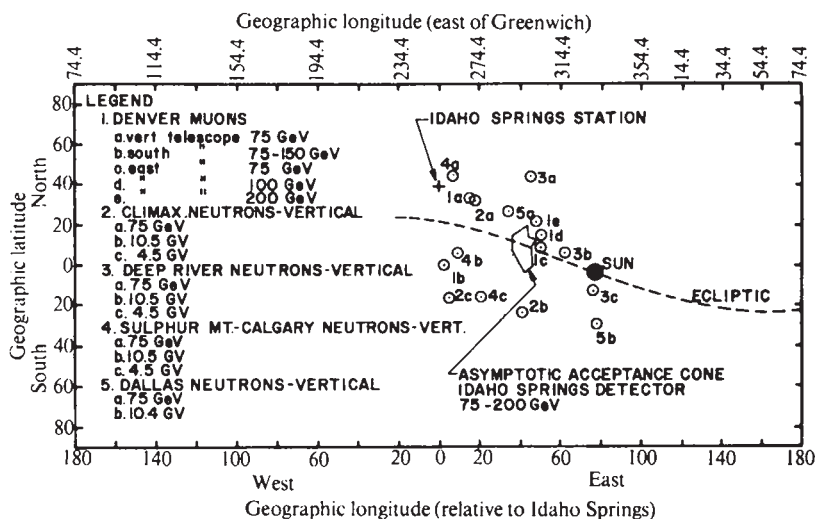
Considering both the lack of an efficient storage mechanism in the near-Sun region¹², and expected minimal scattering effects in the interplanetary region for the particle energies involved in the experiment, our results constitute good evidence for particle acceleration occurring during the initial phase of the solar flare.

This conclusion is consistent with the use of the type IV emission for setting independent upper limits to the particle acceleration period¹, based on the corresponding electromagnetic characteristics of the flare, and also with the identification of the period of filament separation with particle acceleration^{1,12}.

No specific acceleration mechanism is postulated to account for our results at this time, but we note that they are consistent with the upper limiting production energies and corresponding number of particle estimates predicted by the Friedman and Hamberger¹³ formulation of the Petschek wave model¹¹.

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Fig. 3 Ground level detecting conditions which existed at ~1340 UT on October 3, 1971, showing asymptotic viewing directions for various stations including our detector. The asymptotic directions for the indicated energies were calculated using the technique of McCracken *et al.*⁷. Station selection was based on directionality and availability of data.



the $E > 175$ MeV channel (operating at only ~50% efficiency) showed an increase of 2 to 3 times background.

The radio signature of the flare (Fig. 2) does not conform to those generally observed for proton producing flares^{5,6}. A typical null appears in the decimetre to metre region, but the rapid intensity falloff above ~3 GHz effectively eliminates the characteristic U-shaped spectral configuration. Most work done in this area was based on low energy proton data ($E \lesssim 100$ MeV), so it is possible that this spectral effect is related to the very high energy nature of our event.

With the exception of our observation, no intensity increase greater than ~2 standard deviations appeared at any of the ground based stations listed in Fig. 3 (private communications). This can be attributed to the heliographic location of the flare, which, because of propagation conditions in the interplanetary region, is not optimal for normal ground level detection. Small intensity increases were apparent for the individual stations about the period of interest, but none were statistically significant by themselves. In particular, the east telescope of the University of Denver multidirectional array, which partially overlapped our asymptotic cone during the event period, showed an increase of ~2% (or 2σ). A detailed statistical analysis is planned when more particle data have been compiled. The case of single station detection is not unique in the history of solar-related particle events⁸⁻¹¹.

Research Corporation, New York, and the Faculty Improvement Committee of Colorado State University.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Radio Detection of Cygnus X-3

DURING a continuing search for radio emission from X-ray sources with the Westerbork synthesis telescope, we have detected a highly variable radio source near the X-ray location of Cyg X-3 (2ASE 2030+40). Its 1950 position is: $\alpha = 20^{\text{h}} 30^{\text{m}} 37.6 \text{ s} \pm 0.1 \text{ s}$, $\delta = +40^{\circ} 47' 12.7'' \pm 1.5''$.

The observations consisted of four 12 h measurements at a frequency of 1,415 MHz, spanning a period of eight months. Altogether, 80 interferometer baselines ranging in length from 48 to 1,470 m with steps of 18 m were used, and the baseline coverage was such that from a combination of the four measurements a complete synthesis of the brightness distribution in the Cyg X-3 region could be obtained. A profile display of the resultant $1^{\circ} \times 1^{\circ}$ synthesis map is shown in Fig. 1. It illustrates the crowded nature of the field which is part of the well known Cygnus X-radio complex. The extended sources, one of which coincides with the Cyg OB2 (VI Cyg) association and others with H II regions, will be the subject of a forthcoming publication.

The point source at the edge of the 2ASE error box for Cyg X-3 stands out by its three concentric diffraction grating rings which no other object in the field exhibits. These multiple rings would not be present in a complete synthesis map, unless the source had varied greatly during the period covered by the observations.

The strongly variable nature of the source is clear from separate analysis of each of the four 12 h observations. Dates and mean flux densities for these observations are given in Table 1. It appears that over a period of a few months the source has varied by an order of magnitude, whereas none of the other objects in the field showed any sign of variability. Both this extreme variability and the near positional coincidence make us confident that it is the radio counterpart of Cyg X-3.

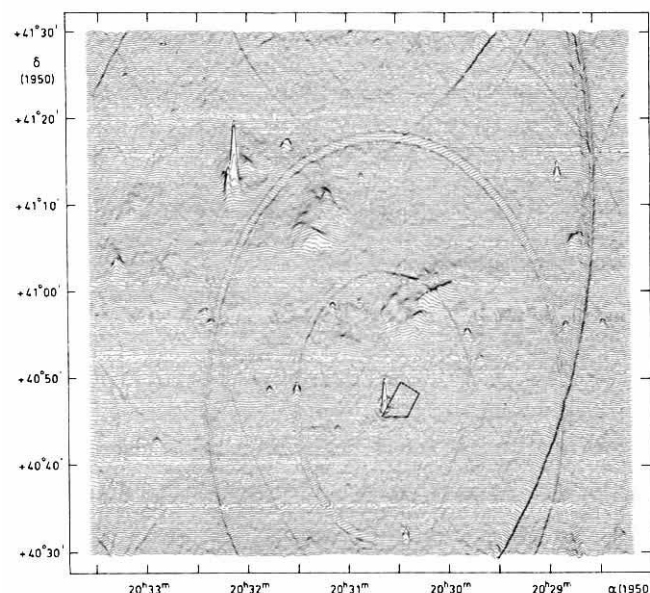


Fig. 1 1,415 MHz brightness distribution in a $1^{\circ} \times 1^{\circ}$ region surrounding Cyg X-3, presented as a series of intensity profiles in right ascension. The quadrilateral marks the 90% confidence limits of the ASE X-ray position from the Uhuru Catalogue.

Table 1 Westerbork 1,415 MHz Data for Cyg X-3

Universal time	Flux density ($10^{-29} \text{ W m}^{-2} \text{ Hz}^{-1}$)	Polarization	
		Linear	Circular
1971 July 19 d 18 h–20 d 06 h	57 ± 2	—	—
1971 Oct. 12 d 13 h–13 d 01 h	23 ± 2	—	—
1972 Mar. 7 d 03 h–7 d 15 h	230 ± 2	< 2%	< 2%
1972 Mar. 9 d 03 h–9 d 15 h	106 ± 2	< 5%	< 4%

From a preliminary analysis it appears that the 1,415 MHz flux density also varies appreciably on a time scale of hours. On March 7, while the source was strongest, the flux decreased by about 30% in less than three hours; during the other days it was relatively steady. In this respect, the behaviour of Cyg X-3 contrasts with that of Cyg X-1 which does not show such rapid variations.

The elements of the Westerbork array have at their focus a pair of crossed dipoles; by taking four cross correlations for each baseline all Stokes parameters of the incoming radiation can be derived. We have thus been able to set stringent upper limits to the mean linear and circular polarization for the days when the Cyg X-3 source was strongest. These mean values are also given in Table 1. It is possible that the source is still highly polarized on a short time scale, and that rapid changes of the position angle and sign during the course of a measurement cause the low mean values. This possibility is being investigated further.

There is no optical object at our radio position on the Palomar Sky Survey prints, but this is not surprising since the area is heavily obscured by the Great Cygnus Rift. From earlier X-ray data, Giacconi *et al.*¹ noted the positional coincidence of Cyg X-3 with the Cyg OB2 association. The radio source lies at the edge of this highly reddened group of stars, and if it is indeed connected with them, its distance would be 2.1 kpc (ref. 2).

With a peak flux density of $230 \times 10^{-29} \text{ W m}^{-2} \text{ Hz}^{-1}$, Cyg X-3 is so far the brightest galactic X-ray object at 1,415 MHz (except for known supernova remnants). The ratio of its radio to X-ray luminosity is about 50 times greater than for Cyg X-1 and exceeds that for Sco X-1 by more than two orders of magnitude. This, together with the unique character of its X-ray spectrum³, suggests that we are dealing here with a completely different type of object. Simultaneous measurements at radio and X-ray wavelengths to investigate possible correlations may provide further insight into its nature.

The Westerbork Radio Observatory is operated by the Netherlands Foundation for Radio Astronomy with the financial support of the Netherlands Organization for the Advancement of Pure Research.

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Radio Observations of Cygnus X-3

Braes and Miley¹, using the Westerbork synthesis array operating at 1,415 MHz, have detected a variable radio source associated with the X-ray source Cyg X-3. This letter reports observations of the radio counterpart of Cyg X-3 at 2,695 and 8,085 MHz with the NRAO interferometer at spacings of 2,400, 1,900 and 500 m.

Data taken from April 29 to May 3, 1972, confirm the presence of a very variable radio source at the following position

Radio source: $\alpha_{1950} = 20^{\text{h}} 30^{\text{m}} 37.63 \pm 0.03 \text{ s}$

$\delta_{1950} = +40^{\circ} 47' 12.5'' \pm 0.5''$

which is 2.1 arc min from the position of Cyg X-3 as recently determined from UHURU satellite data²

X-ray source: $\alpha_{1950} = 20^{\text{h}} 30^{\text{m}} 29 \text{ s} \pm 12 \text{ s}$

$\delta_{1950} = +40^{\circ} 47' 24'' \pm 2''$

therefore the variable radio source is well within the error box of the X-ray position. At all times the radio source was an unresolved point source, therefore an upper limit of 0.5 arc s can be placed on its angular diameter.

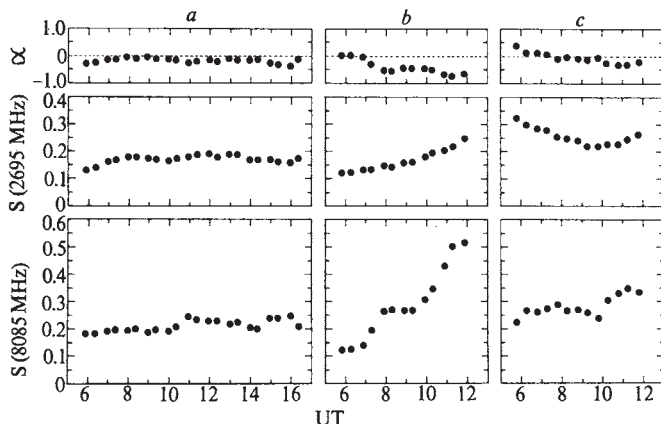


Fig. 1 Spectral index and flux densities at 2,695 and 8,085 MHz are plotted as functions of Universal Time for Cyg X-3 on May 1 (a), 2 (b), and 3 (c), 1972. The flux densities are measured in flux units. The error limits on the flux densities are the size of the filled circles.

The results of the observations at 2,695 and 8,085 MHz are presented in Table 1, where the first three columns give the time of observation and the flux densities at the two frequencies. Each entry represents a 25 min average and the error limits on the flux densities are ± 0.01 f.u. (1 f.u. = $10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$). To illustrate the very important relationship between the flux densities and the spectral index, α , calculated from $\alpha = -\ln [S(8,085)/S(2,695)]/\ln (8,085/2,695)$, these quantities are plotted in Fig. 1 as a function of time for the data on May 1, 2 and 3, 1972. Cyg X-3 is clearly the strongest of the presently known X-ray stars at these radio frequencies and, unlike Sco X-1^{3,4}, the source is strongest at high frequencies most of the time. With the exception of 2 h on May 3, 1972, the Cyg X-3 radio counterpart behaves as expected for a variable thermal radio source. At the present time, the only known radio sources with similar behaviour are the radio binary stars, particularly β Persei (Algol)^{5,6} and β Lyrae^{5,6}. The similarity between the radio data of Cyg X-3 and β Persei is very obvious. The flux density and spectral index variations of Cyg X-3 on May 1, 2 and 3 are strikingly similar to the behaviour of β Persei⁶ on Jan. 14/15, Jan. 16/17, and Jan. 22/23, 1972, respectively. The only sign of non-thermal behaviour in β

Persei on Jan. 22/23 is like that of Cyg X-3 on May 3. This empirical similarity in time scale, spectral index variation, and so on, strongly suggests that the physical mechanisms responsible for the radio emission in Cyg X-3 and β Persei are the same. It has been argued⁷ that the β Persei radio source is primarily a hot thermal bremsstrahlung source which should be a transient X-ray source.

Table 1 Data and Thermal Source Parameters for Cygnus X-3

Universal time	S_{ν} 2,695 [$10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$]	8,085 [$10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$]	$E T_e^{-1.45}$ [$\text{pc cm}^{-6} \text{ K}^{-1.45}$]	$T_e \Omega_s$ [K sr]	$E \Omega^{1.45}$ [$\text{pc cm}^{-6} \text{ sr}^{1.45}$]
April 29, 1972					
1226	0.120	0.180	31	0.78×10^{-6}	4.3×10^{-8}
April 30, 1972					
1224	0.250	0.370	30	1.7	12.0
May 1, 1972					
0552	0.129	0.181	26	0.93	4.7
0620	0.136	0.183	23	1.1	5.0
0655	0.157	0.192	16	1.5	6.1
0720	0.166	0.197	14	1.8	6.7
0755	0.177	0.194	9	2.8	7.9
0820	0.177	0.199	11	2.4	7.6
0855	0.174	0.186	8	3.2	8.0
0920	0.169	0.196	13	2.0	6.9
0955	0.164	0.193	14	1.8	6.6
1020	0.172	0.209	16	1.7	7.0
1055	0.178	0.244	24	1.3	7.4
1120	0.185	0.234	19	1.7	7.7
1156	0.190	0.229	15	2.0	8.1
1220	0.176	0.227	20	1.5	7.2
1256	0.189	0.217	12	2.3	8.2
1320	0.188	0.223	14	2.0	8.0
1356	0.167	0.203	16	1.7	6.7
1420	0.169	0.199	14	1.9	6.9
1456	0.169	0.236	26	1.2	6.9
1520	0.161	0.236	29	1.1	6.6
1556	0.156	0.246	35	0.96	6.5
1620	0.174	0.206	14	1.9×10^{-6}	7.2×10^{-8}
May 2, 1972					
0548	0.121	0.123	4	3.6×10^{-6}	5.6×10^{-8}
0616	0.123	0.125	4	3.7	5.7
0651	0.132	0.141	8	2.4	5.4
0716	0.135	0.196	28	0.92	5.1
0751	0.146	0.266	47	0.79	6.6
0816	0.144	0.270	49	0.77	6.7
0851	0.159	0.267	40	0.92	7.0
0916	0.160	0.269	40	0.92	7.1
0951	0.180	0.306	41	1.0	8.5
1016	0.195	0.345	44	1.1	9.8
1051	0.202	0.429	61	1.0	12.2
1116	0.217	0.503	70	1.0	14.8
1151	0.247	0.515	59	1.2	16.1
May 3, 1972					
0544	0.321	0.221			
0612	0.294	0.267			
0648	0.282	0.259			
0712	0.276	0.271			
0748	0.249	0.286	12	3.1	12.0
0812	0.244	0.264	8	4.1	13.0
0848	0.238	0.270	11	3.1	12.0
0912	0.216	0.258	15	2.3	9.7
0948	0.216	0.237	9	3.3	10.0
1012	0.222	0.302	24	1.7	10.0
1048	0.225	0.326	28	1.5	11.0
1112	0.240	0.347	28	1.7	12.0
1148	0.259	0.332	20	2.2×10^{-6}	13.0×10^{-8}

If the dominant radio emission mechanism for Cyg X-3 is thermal bremsstrahlung, we can solve for some of the parameters of the source. The radio flux density can be expressed as⁷

$$S_{\nu} = \frac{2k}{\lambda^2} T_e \Omega_s [1 - \exp(-0.28 E T_e^{-1.45} \nu^{-2.05})] \quad (1)$$

where k = Boltzmann constant, ν = frequency (GHz), λ = wavelength, T_e = electron temperature, Ω_s = source solid angle, and E = emission measure.

Simultaneous measurements of S_ν at 2,695 and 8,085 MHz allow the determination of $T_e\Omega_e$ and $E T_e^{-1.45}$ as functions of time whenever the argument in the exponential in equation 1 is large enough. By multiplying $E T_e^{-1.45}$ by $T_e\Omega_e$, raised to the 1.45 power, a parameter independent of T_e , $E \Omega_e^{1.45}$ can be obtained. The results of such an analysis, whenever it was possible, are presented in Table 1. Only the first two hours on May 3 are not compatible with a thermal source model. We conclude from this that, just as Sco X-1 occasionally shows signs of a rare thermal component⁴, Cyg X-3 contains both thermal and non-thermal components, with the non-thermal behaviour being rare.

A few simple conclusions can be drawn from the thermal source parameters derived in Table 1. First, as has previously been noted for β Persei (R. M. H., manuscript submitted), $T_e\Omega_e$ is roughly constant, except for variations as expected from the noise in the flux density data, on any particular day; for example, $T_e\Omega_e = 1.9 \times 10^{-6}$ K sr on May 1, 1.0×10^{-6} on May 2, and 2.6×10^{-6} on May 3. The most interesting differences occur in $E \Omega_e^{1.45}$ which was roughly constant at 7.0×10^{-8} on May 1 and 1.1×10^{-7} on May 3; however, there was a clear variation from 5×10^{-8} up to 1.6×10^{-7} during the flare on May 2, 1972. If the thermal interpretation of the radio data for Cyg X-3 is valid, simultaneous measurements at radio and X-ray energies should provide an unusual opportunity to look for correlations and to further determine the parameters of the source.

We thank Giacconi *et al.*² for providing a copy of the UHURU X-ray source catalogue in advance of publication. The National Radio Astronomy Observatory is operated by Associated Universities Inc. under contract with the US National Science Foundation.

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Received May 31, 1972.

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LR Cen is not Cen X-3

THE eclipsing variable LR Cen has been suggested^{1,2} as a candidate for the optical counterpart of Cen X-3³ on the basis of the near coincidence of the position and period of LR Cen with those of Cen X-3. My observations⁴ and those of Kristian *et al.*¹ show that in March, the time of eclipse of the secondary of LR Cen nearly coincided with that of Cen X-3. Also LR Cen and Cen X-3 both have orbits of low eccentricity; thus, LR Cen and Cen X-3 have similar positions, periods, phases and eccentricities.

The difference in periods, however, though small, is significant. The observed eclipses of the X-ray source would have occurred at several different phases in the cycle of LR Cen if its period was unchanged since the original determination of 2.095595 ± 0.000009 days by Uitterdijk⁵ in 1932. I have redetermined the period of LR Cen from my observations of its primary eclipse on March 16, 1972 (Fig. 1a) and from those obtained on April 26, 1972 (Fig. 1b). The precise times of the minima were determined from the coefficients of second order polynomials which were fitted to the observations using the method of least squares. The value for the period of LR Cen of 2.0954 ± 0.0007

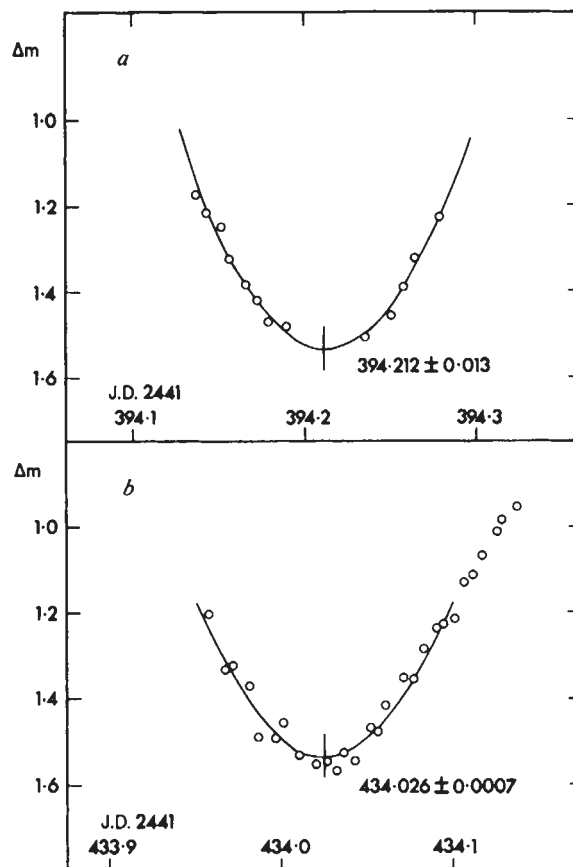


Fig. 1 Primary minima of LR Cen in V, given in magnitudes with respect to Uitterdijk's comparison star C. The lines represent the second order polynomials used to determine the time of the minima.

days was obtained for the 19 cycles elapsed between the primary minimum on March 16, 1972 (J.D. 244 1394.212 $\pm$ 0.013) and the primary minimum on April 26, 1972 (J.D. 244 1434.026 $\pm$ 0.007). These observations show that the period of LR Cen is unchanged, and that it is significantly different from 2.08712 ± 0.00004 days, the period of Cen X-3.

Thus, the near coincidence of the times of eclipse found in March is wholly coincidental, and I now conclude that LR Cen is not the optical counterpart of Cen X-3.

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Ring Current Effect on Magnetospheric Electron Density Profiles derived from Plasmopause Whistlers

THE longitudinal drift of van Allen radiation belt charged particles, with opposite charges drifting in opposite directions in the geomagnetic field, constitutes a westward directed ring

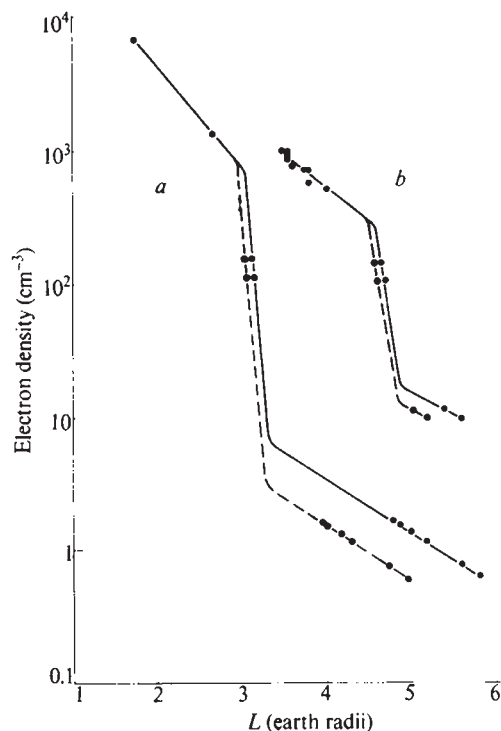


Fig. 1 Electron density profiles in the magnetospheric equatorial plane derived from multi-flash one-hop whistlers recorded on two different occasions during 1970. *a*, Chilbolton ($L \sim 2.4$), March 8, 1970, 1703 UT, $K_p = 8^-$. *b*, South Uist ($L \sim 3.4$), July 2, 1970, 1945 UT, $K_p = 1^-$. On each occasion the plasmapause, the marked knee in the profile where the electron density decreases by a factor $\gtrsim 20$ within a fraction of an Earth radius, is evident. ---, Calculated profiles, as discussed in the text, using appropriate models for the reduction of the geomagnetic field due to the ring current^{4,5}; —, profiles presented earlier³ assuming the geomagnetic field to be that of a dipole.

current at a distance of several Earth radii¹. Although at magnetically quiet times this current scarcely neither reduces the geomagnetic field nor affects the parameters that determine the group velocity of a whistler mode signal propagating along a particular field line, these can be significantly changed at magnetically active times, for example during the main and recovery phases of a magnetic storm, when the ring current is stronger².

The assumption that is conventionally made when deriving magnetospheric electron density profiles from whistlers, that the Earth's magnetic field is a perfect dipole, is valid during magnetically quiet periods. Electron density profiles exhibiting the plasmapause have thus been deduced from whistlers observed for the first time in the UK at times of both very high and low planetary magnetic index³. During geomagnetic storms, however, the enhanced ring current reduces the equatorial magnetic field strength, and so reduces the electron gyrofrequency and the nose frequency of a whistler propagating along a field line that passes through a particular point on the

Earth's surface. It is necessary, therefore, to include both the distortion of the field line due to the ring current and the change of the whistler mode refractive index when determining the equatorial electron density along the actual path of propagation.

In this letter we present results which show quantitatively the effect of the ring current perturbation to the geomagnetic field on whistler propagation near the plasmapause, using computations performed by Sagredo⁴ and Sagredo and Bullough⁵.

On two occasions, at 1703 UT on March 8, 1970 ($K_p = 8^-$) and at 1945 UT on July 2, 1970 ($K_p = 1^-$), multi-path whistlers have been received and analysed. For each date, two equatorial electron density profiles are shown in Fig. 1, one corrected for the ring current effect and the other uncorrected. In each case, within the plasmasphere, we used a diffusive equilibrium plasma distribution model (unpublished); outside the plasmapause we used a collisionless (R^{-4}) model.

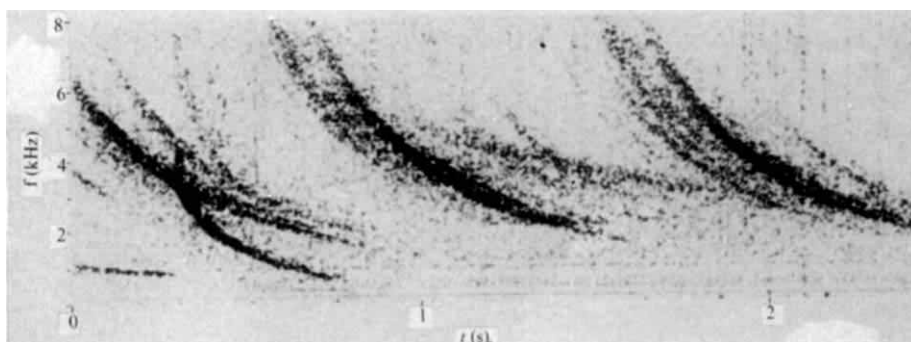
On the earlier occasion, the curve presented by Sagredo and Bullough⁵ for a very severe geomagnetic storm with a peak kinetic energy density (E_0) of charged particles of 900 keV cm^{-3} , with a corresponding depression of the Earth's magnetic field in the equatorial plane (D_{ST}) of -128γ , is appropriate for the correction of the electron density profile obtained from the whistler data. During this magnetic storm, the peak differential intensity of $0.31 \leq E_p \leq 0.45 \text{ MeV}$ geomagnetically trapped protons at the Injun 5 orbit increased by a factor of up to ~ 20 (ref. 6). The magnetometer aboard OGO-6 at 1,050 km altitude at the equator measured a magnetic disturbance, at 1940 LT at the satellite (close to the UT of the whistler observation), of about -135γ (ref. 7).

On the later occasion, the weakest of the four ring current models presented by Sagredo and Bullough⁵, with $E_0 = 150 \text{ keV cm}^{-3}$ and with $D_{ST} = -25 \gamma$, is used. This model is appropriate because, on the day on which the observations were made, the eight three-hourly K_p indices were 1^+ , 2^+ , 4^+ , 3^+ , 2^+ , 2^+ , 1^- , and 1^+ .

From an examination of the equatorial electron density profiles (Fig. 1), it is clear that the ring current effect is pronounced outside the plasmapause, noticeable on the plasmapause and insignificant within the plasmasphere. The effect of the ring current modelled for the recovery phase of a magnetic storm is a reduction of the plasmapause distance by $0.08 \text{ Earth radii } (R_E)$ from its dipole field value. Comparing the profiles shown in Fig. 1, we see that the equatorial electron densities at $L \sim 5$ are reduced by a factor ~ 2.2 and ~ 1.3 when the effects of intense and weak ring currents, respectively, are taken into account. When the effect of a ring current is allowed for, the electron density change at the plasmapause is increased, making the plasmapause an even more noticeable feature of the distribution of thermal plasma in the magnetosphere. This result agrees with direct satellite measurements of huge electron density gradients at the plasmapause⁸.

It is also important to note that outside the plasmapause the L value of the field line yielding a particular equatorial electron density value is shifted inwards by $\sim 0.8 R_E$ when the ring current is strong and $\sim 0.4 R_E$ when weak. The reception of nose whistlers, with nose frequencies as low as $\gtrsim 4 \text{ kHz}$ (Fig. 2), at a comparatively low latitude site ($L \sim 2.4$) is thus

Fig. 2 Dynamic spectrum of multi-flash, multi-path one-hop whistlers recorded at Chilbolton, Hampshire ($L \sim 2.4$), at 1703 UT on March 8, 1970, during a severe magnetic storm.



understood as being primarily a result of the reduction of the electron gyrofrequency brought about by the ring current. This effect also reduces the range of L -values immediately beyond the plasmapause where there are no points on the electron density profile, presumably because there are no whistler ducts there. On the quieter occasion, this L -value range is reduced essentially to zero; during the magnetic storm, it is reduced by a factor ~ 2.4 to only $\sim 0.6 R_E$. In general, the inclusion of the ring current effect enables the position of the plasmapause to be specified more accurately.

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Super-resolution Aperture Scanning Microscope

THE classical theory of the resolving power of optical instruments implies a limit to the observation of details in an object if these are significantly smaller than one wavelength, λ_0 , of the illuminating radiation. This Abbe barrier is not entirely impenetrable. Lukosz^{1,2} has shown how an improvement by a factor of two can be made by the use of complementary spatial filters. Attempts to proceed further in this direction

are soon frustrated, however, because the spatial frequencies one is seeking to transfer are such that the waves become evanescent in the direction in which one would like them to propagate. Nassenstein has developed an ingenious scheme³ in which evanescent waves are used to illuminate the object, and a magnified image is obtained using a holographic technique. The resolution capability is determined by the wavelength of the evanescent wave. This is less than λ_0 , but it is not easy to devise systems where it would be very much smaller.

Such approaches depend on transferring information from all the resolvable points in the object plane to the image plane simultaneously—that is, a parallel processing system. The problem of obtaining super-resolution can be simplified greatly by resorting to a scanning technique. If we can illuminate one resolution area of the object at a time, the problem of extracting the information becomes relatively simple. One cannot overcome the classical resolution limit simply by focusing a beam of radiation on to the object, because the minimum, diffraction-limited spot size is also of the order of one wavelength. If, however, we can fabricate a small aperture of radius r_0 , ($r_0/\lambda_0 \ll 1$), in a thin diaphragm, we can obtain information about a comparably small object region—provided only that the object is also located very close to the aperture. These considerations lead to a simple system (Fig. 1).

The illumination system is shown in the form of an open resonator. Because the aperture area is only $(r_0/w_0)^2$ of the beam area, where w_0 is the beam radius, a signal which is very small compared with the illuminating power must be used to obtain a significant improvement over the classical resolution. Moreover, this signal is superimposed on a much larger background radiation so that the "contrast" will be very poor. This deficiency can be overcome by vibrating the object at a frequency f_m . The required signal is then "tagged" by this modulation frequency and is readily separated in the receiver system.

The object is illuminated by the fields existing in the neighbourhood of a small aperture in a thin diaphragm. Our interest is confined to apertures which are significantly less than one wavelength in diameter, so the field on the object side of the diaphragm ($z > 0$) can, with good accuracy, be approximated by a pair of magnetic and electric dipoles⁴. If the diaphragm is illuminated normally (Fig. 1) the magnetic dipole will predominate. The aperture also gives rise to a dipole field in the region $z < 0$ above the diaphragm; the strength and orientation of this field will contain the desired information. A dipole radiating into a half space has a very broad radiation pattern. But in the situation of Fig. 1 the dipole radiates into an open resonator; if Q is sufficiently high, the radiation power is predominantly transferred to the resonant mode⁵.

The sources of object contrast are found in the distribution of the electric and magnetic permittivities and in the distri-

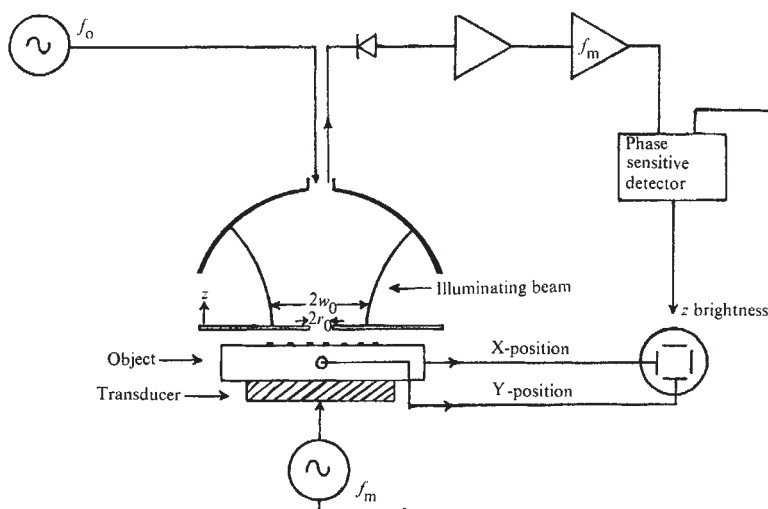


Fig. 1 Basic arrangements of scanning microscope. f_0 , illuminating frequency; f_m , vibration frequency; w_0 , radius of illuminating beam; r_0 , radius of aperture.

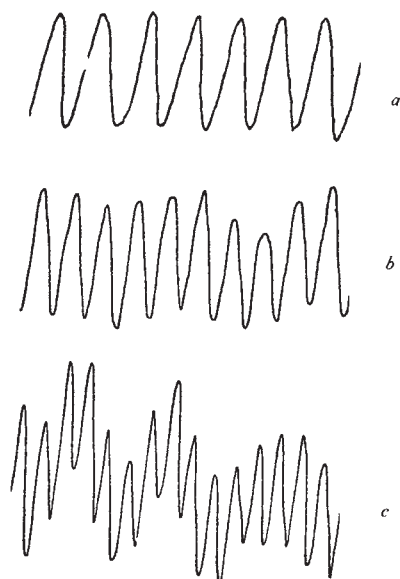


Fig. 2 Resolution of metal gratings: *a*, line width 1.0 mm; *b*, line width 0.75 mm; *c*, line width 0.5 mm.

bution of the conductivity, which alter the energy stored and the energy radiated. If there are significant gradients of these quantities within the dipole field, there will also be a contribution to the signal arising from the distortion of the dipole field. One effect of such distortion which could be particularly useful in subsequent signal processing is a rotation of the polarization of the perturbed dipole field. Changes in the energy stored will give rise to a shift in resonator frequency; changes in the radiation will also perturb Q , but this effect is much smaller.

In order to obtain some estimate of the signal strength, we have calculated the frequency perturbation caused by a metal object which is vibrated so as to close the aperture at one part of the cycle, and which is removed, effectively outside the range of the dipole near-field, half a cycle later. This calculation has been carried out using simple perturbation theory, assuming that the change in resonator frequency δf is given by

$$\frac{|\delta f|}{f_0} = \frac{|U_E - U_H|}{U_{\text{RES}}}$$

where U_E and U_H are the electric and magnetic energies stored in the near field of the aperture, and U_{RES} is the total energy stored in the open resonator. If the resonator is tuned to the optimum frequency (displaced from the resonant frequency by a fraction $3^{1/2}/Q$), the signal power, modulated at the frequency f_m , takes the form

$$S \simeq \frac{6 \times 10^{-2} (k_0 r_0)^3}{\alpha (k_0 w_0)^2} P_{\text{in}}$$

where α is the fraction of energy lost at each mirror per reflexion, P_{in} is the power available from the illuminating source and $k_0 (=2\pi/\lambda_0)$ is the wave number. If $\alpha=10^{-2}$, $w_0=\lambda_0/2$ and $r_0=\lambda_0/20$ the signal power is $0.018 P_{\text{in}}$. This result refers to an ideal object but it is nevertheless encouraging, and suggests that it should be possible to work with even smaller apertures.

Although the signal power is enhanced by the use of high reflectivity mirrors, it is not otherwise dependent on Q . Short, low Q cavities can therefore be used, which reduces the demands on the frequency stability of the illuminating source.

We sought to demonstrate the principle in experiments carried out at a frequency close to 10 GHz ($\lambda_0=3$ cm). The illuminating open resonator had a radius of curvature of 10 cm, and was positioned 9.3 cm from the aperture plane. A conventional waveguide hybrid T bridge was used to separate

the incident from the reflected waves. The receiving system consisted of a simple detector followed by a low noise amplifier and an amplifier tuned to f_m . This signal was then compared in a phase sensitive detector supplied with a reference from the acoustic modulator source.

The object was mounted on a vibrator attached to an XY table, fitted with position transducers. The output signal on scanning the system was displayed on an XY recorder, or on an oscilloscope, using a brightness modulation display. Test objects were prepared using patterns of aluminium deposited on glass slides. Fig. 2 shows a scan across a grating with line and gap width 1 mm, 0.75 mm and 0.5 mm, in each case using an aperture diameter $2r_0=1.5$ mm. In the third case a grating with a line width of $\lambda_0/60$ is clearly resolved.

Fig. 3 shows an oscilloscope photograph of a two dimensional scan, compared with the original. The width of the lines of the letters is 2 mm ($\lambda_0/15$).

In the use of apertures which are small compared with one wavelength it is meaningful to distinguish between electric and magnetic contrast. It was therefore of interest to see whether the microscope could record a spatial variation of the relative dielectric permittivity. In this experiment we chose the aperture fields to correspond to a purely magnetic dipole, for which the near-field stored electric energy is very small, so that the signal would arise predominantly from gradients in the distribution. Fig. 4 shows a scan from the edge of a 'Perspex' sample $\epsilon_r=2.58$ and across a joint to a polythene sample $\epsilon_r=2.24$. The border between the two materials is clearly resolved.

Our initial aim in carrying out the microwave experiment was to convince ourselves, and hopefully others, that the basic concept is sound. The encouraging experience with the system has, however, led us to reconsider the possibility of applications within the microwave band. By raising the illumination power above the 1 mW used in these experiments, and by using more sophisticated receiver systems, it would appear that one could gain an extra 30 dB in signal strength. This suggests a resolution capability of the order of a few tens of microns at 8 mm. Without going to the ultimate in resolu-

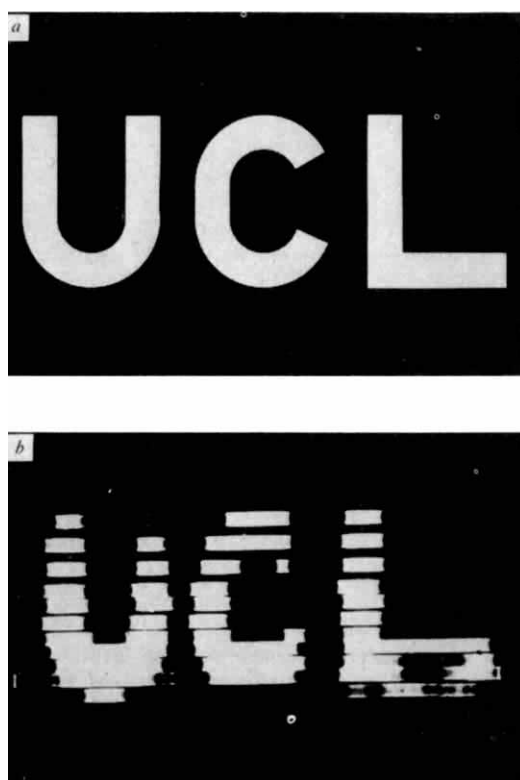


Fig. 3 Imaging of metal characters. *a*, Original; *b*, image.

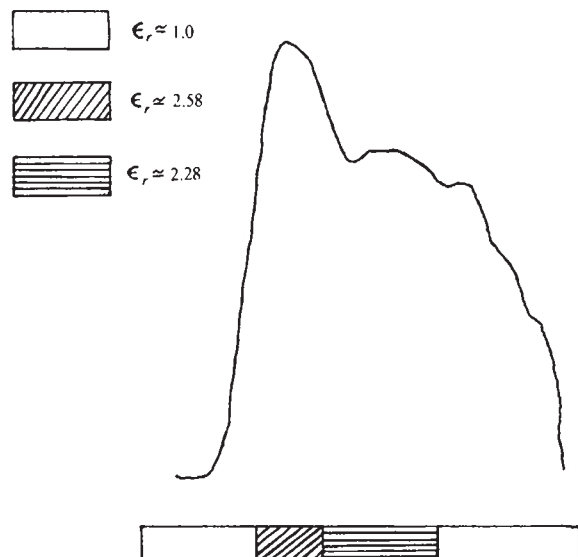


Fig. 4 Imaging of dielectric sample.

tion capability we see, as one possible application, the testing of substrate materials to display small variations in the electric or magnetic properties.

At the other end of the spectrum one might hope to build a super-resolution optical microscope. There is no inherent difficulty, using electron beam techniques, in making apertures in a sufficiently thin metallic film which are much smaller than the optical wavelength. The need to place the object within $\sim r_0$ from the aperture implies that one would be limited to the observations of objects which can be prepared with a flatness significantly better than an optical wavelength.

Although the optical microscope may not be beyond reach, in our view a more immediately hopeful application is the construction of an infrared microscope with a resolution comparable with that normally attainable in the visible optics spectrum.

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BIOLOGICAL SCIENCES

Liquid Phase Synthesis of Peptides

ONE of the most exciting recent developments in peptide synthesis is the introduction of the solid phase method¹ which facilitates rapid synthesis of peptides by the elimination of tedious purification steps. Excess reagents are removed by filtration. The ease of these operations led to speculations that the method might be used for the synthesis of enzymes, but careful investigation has shown that the individual coupling steps are not quantitative, so that erroneous sequences accumulate, together with the desired polypeptide^{2,3}. General methods to separate such complex mixtures are still not available,

especially if the chain length exceeds 20 amino-acids, so much was clear in the attempted synthesis of the enzyme ribonuclease⁴ and other larger peptides. Another disadvantage of the solid phase method compared with synthesis in solution is the restriction in the choice of solvents, coupling methods and protecting groups.

The most effective solution to these problems would be to force the coupling reaction to completion; but since the solid phase method is a heterogeneous reaction, this is bound to be more difficult than in "classical" peptide synthesis in solution. Improvements have been made by increasing mass transfer on the solid support and, for example, glass pellets have been coated with a thin layer of chloromethylated polystyrene². Unfortunately the resin layer on the glass surface is mechanically unstable and it is difficult to perform a synthesis requiring more than thirty coupling steps. Better stability is achieved if a hydroxymethyl-benzene group is covalently bound to silica by reaction of *bis*-(hydroxymethyl)-benzene with the silanol groups of any type of silica, for example, glass beads, silica gel or quartz⁵⁻⁷. The silicic ester with one hydroxylic group is formed and the other hydroxymethyl group protrudes from the surface, rather like a brush, and the carboxylic group of the first C-terminal amino-acid can be esterified to the hydroxymethyl group⁵. (In high speed chromatography these "brushes" are highly ordered and allow fast diffusion and mass transfer^{6,7}.) But failure sequences are still observed, especially if amino-acids such as valine or isoleucine are present which are known to couple poorly. Steric factors are responsible for this and further improvements are impossible.

The principal advantage of the solid phase method is the application and easy removal of excess reagents during the coupling step and the possibility of mechanization. From every other aspect, however, synthesis in solution would be more convenient. In attempts to use soluble non-cross-linked polystyrenes for peptide synthesis⁸ separation from the reagents after coupling, accomplished by precipitation of polystyrene, was incomplete because of coprecipitation.

We have developed a liquid phase method for peptide synthesis which combines the advantages of the "classical" synthesis in solution with those of the solid phase method⁹. A polymeric protecting group is bound to the carboxylic group of the C-terminal amino-acid so as to endow the growing peptide chain with good solubility. Polyethylene glycol is very useful for this purpose, especially for synthesis in aqueous solution, and various other polymeric groups can be used, for example, polyvinyl alcohol or polyvinylamine copolymerized with polyvinylpyrrolidone, according to the requirements of solvents and coupling methods. Besides solubility, the polymeric group has the advantage that from the beginning of a synthesis the reagents and the protected growing peptide differ in molecular size, so that separation of the growing peptide chain is possible at every coupling step. The easiest way to achieve this separation is dialysis by membrane filtration¹⁰.

Thus the advantages of solid phase synthesis are preserved, with the further advantage that the growing peptide chain can be purified as often as desired without cleavage from the polymer support. Normal filtration is replaced by ultrafiltration, which is achieved in homogeneous solution. All the experience of "classical" peptide synthesis in solution can be applied to this method and an effective separation can be achieved if required by a poor coupling step. The polymeric protecting group need not be split off to do this. This procedure gives considerable freedom of choice of coupling procedures, solvents, protecting groups and working conditions. Some features and advantages of the new method can be summarized as follows. (1) Synthesis achieved in homogeneous solution and separation from excess reagents is quick and easy by membrane filtration without the tedious separations of "classical" peptide synthesis. (2) Better yields are obtained by reaction in homogeneous solution compared with the Merrifield synthesis with heterogeneous phases. (3) Most known protecting groups and coupling methods can be used. (4) The polymeric protect-

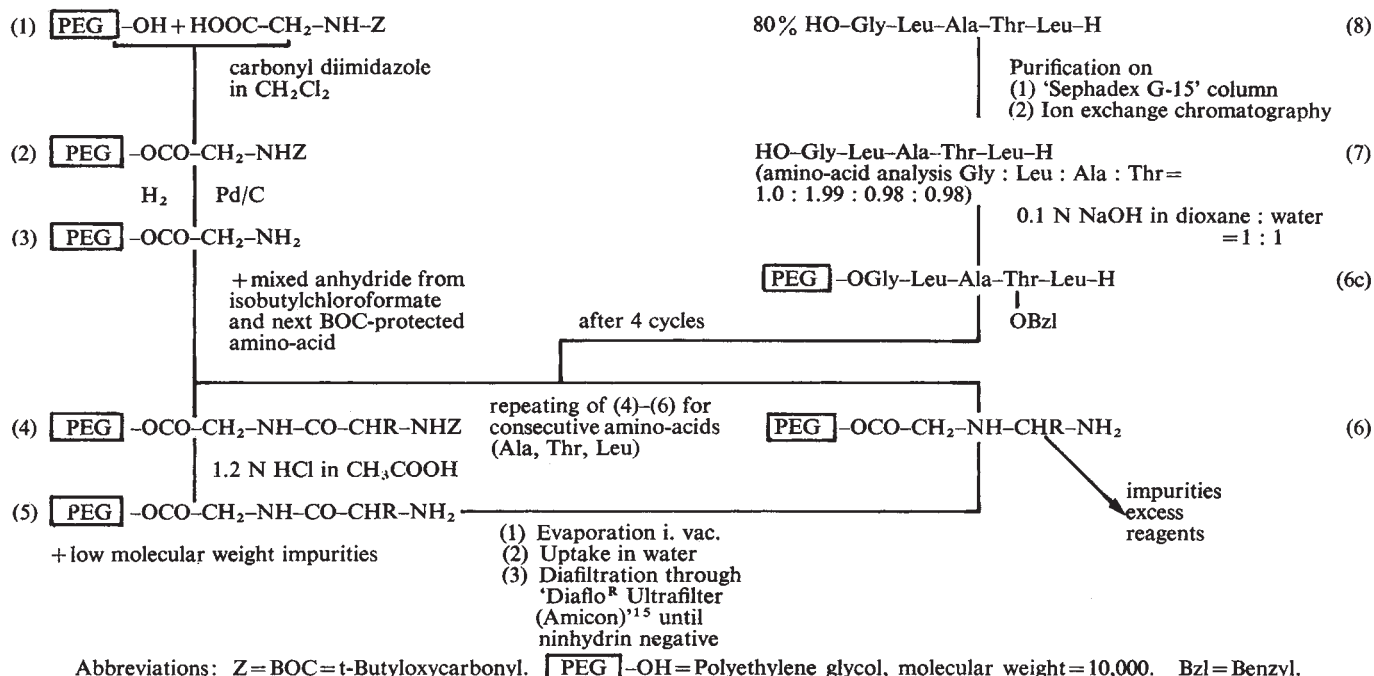
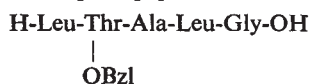


Fig. 1 Flow chart for the synthesis of HO-Gly-Leu-Ala-Thr-Leu.

ing group can be designed according to the requirements of molecular size and solubility. (5) The desired compound can be separated from failure sequences in critical intermediate coupling steps. (6) The completeness of the coupling can be controlled by taking aliquot volumes. Monitoring the ^{19}F -nuclear magnetic resonance of protecting groups or "fluorine sensor"^{11,12} groups is especially useful for analytical control. (7) Scaling up of the reaction is easier in solution. (8) Automation is easy and a synthesizer apparatus has been designed. (9) In general all procedures of "classical" peptide synthesis can be used. If a coupling proceeds with low yields this step can be repeated using another coupling method. (10) The peptides synthesized by this method can be subjected to fragment condensation without splitting off the C-terminal polymeric protecting group. The solubilizing effect of the polymeric protecting group overcomes the most critical problem of fragment condensation, the limited solubility of medium size peptides.

The synthesis of the pentapeptide



using BOC-amino-acids and the mixed anhydride method¹³ for coupling and for the tetrapeptide is outlined in the flow chart (Fig. 1).

The tetrapeptide H-Ile-Ala-Pro-Val-OH has also been synthesized in methylene chloride using dicyclohexyl-carbodiimide as coupling reagent instead of the mixed anhydride method. The tetrapeptide was split off the polymer by transesterification with a 1 M solution of sodium methylate in methanol. Purification was carried out as described in the flow chart (amino-acid analysis Val-Pro-Ala-Ile = 1.0 : 1.0 : 0.98 : 0.99).

Each coupling step in both syntheses was tested by the ninhydrin¹⁶ and the dansylchloride¹⁷ method and gave better yields than 99%. Both peptides were investigated by the ^{19}F nuclear magnetic resonance method and did not show any failure sequences. No racemization could be detected by gas chromatography.

Various other peptides have been synthesized by this method using polyethyleneglycol and other polymer carriers. These results will be published later.

The liquid phase method opens up new possibilities for the synthesis of enzymes both by the step-wise synthesis and by the fragment condensation strategy.

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Ouabain and the Onset of the Blood-Brain Barrier in Neonate Chicks

SMALL doses of ouabain have powerful central nervous depressant effects and induce hypothermia when administered into the cerebral ventricles of mice and rats¹⁻³, in sharp con-

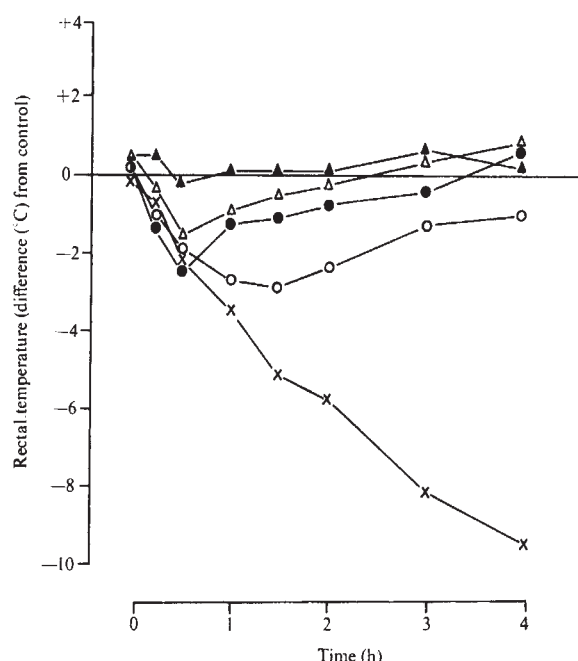


Fig. 1 Effect of age of young chicks on the response to peripherally-administered ouabain. Chicks received 0.7 mg/kg ouabain intraperitoneally (x) 2 days, (o) 3 days, (●) 4 days, (△) 5 days or (▲) 10 days after hatching. In each case controls received 10 ml./kg saline intraperitoneally.

trast to the excitatory and convulsant effects produced by larger doses given centrally or peripherally in rodents²⁻⁵. To obtain further evidence that the depressant effects result from a direct action on the central nervous system we have used young chicks, in which adult blood-brain characteristics are not reported to be present until about the fourth week of life^{6,7}.

Ranger chicks, housed in large wire cages in groups of at least twenty-four, were maintained at a laboratory temperature of 33°–34° C. Two hours before each experiment they were transferred to an air-conditioned room maintained at 21 ± 1° C. Rectal temperature was used as an indication of core temperature, each estimation representing the mean of five animals.

Ouabain (0.2–1.0 mg/kg intraperitoneally) produced hypothermia and central nervous depressant effects similar to those seen after intracerebroventricular injection of 0.1–0.4 µg in the mouse. In 3-day-old chicks these effects were dose-dependent for the range 0.2–0.5 mg/kg intraperitoneally the highest dose producing a maximum decrease in body temperature of 3° C within 1 h of injection, however, the magnitude of the response varied markedly with the age of the chick. In 2-day-old chicks ouabain (0.5 mg/kg intraperitoneally) produced a much greater degree of hypothermia, accompanied by muscular weakness, incoordination and ptosis. The chicks assumed squatting posture with wings and beak touching the floor, vocalization stopped, they were not easily aroused by handling and retching and vomiting developed within 1 h of injection. The sensitivity to ouabain decreased rapidly with age, until at ten days after hatching, no effects either on behaviour or body temperature could be produced using doses up to 0.7 mg/kg intraperitoneally (Fig. 1).

These experiments provide further evidence for a potent central, depressant effect of ouabain. We are particularly interested in the rapidity with which insensitivity to these effects develops with the age of the chick. By comparison, the change in response with maturation to some other agents, including certain of the sympathomimetic amines⁸, does not take place until about the fourth week. It seems likely that the blood-brain barrier develops at different rates for different compounds depending partially on the molecular structures of

those compounds. Changes in the integrity of the blood-brain barrier may also reflect the development of fully functional neuro-transmitter systems within the brain. It is thus impossible to define the degree of maturity of the blood-brain barrier in the chick unless it is related to specific pharmacological agents.

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Experimental Infection of Chimpanzees with the Virus of Hepatitis B

THE effects of viral hepatitis in chimpanzees are similar to those in man^{1,2}, and the disease can be transmitted from chimpanzees to humans³. Hepatitis B or Australia antigen (HBAG) has been reported in healthy chimpanzees⁴ and we have demonstrated antibody (HBAb) in 55% of these animals by radioimmunoassay. We have also shown the immunological identity between human and chimpanzee HBAG and HBAb⁵. Two susceptible chimpanzees (Xat and Yon) were inoculated intravenously with 1.0 ml. of a 1 : 10 dilution of human plasma containing HBAG and known to cause hepatitis B in humans. The animals were bled three times weekly and liver biopsies were obtained twice monthly in order to study immunological, biochemical, and histological responses. To determine HBAG, matrix gel diffusion (MGD)⁶, complement fixation (CF)⁷, radioimmunoassay (RIA)⁸ and passive haemagglutination (HA)⁹ techniques were used. To detect HBAb, the CF and MGD tests were used in addition to a "solid phase direct" radioimmunoassay system. Chimpanzee susceptibility to hepatitis B virus was inferred from a lack of demonstrable HBAb in serum by both HA and RIA techniques. Serum titre of HBAb in the diluted human plasma inoculum was 1 : 128 by the CF technique.

Xat developed a transient antigenaemia 70 days after inoculation, with development of antibody 36 days later. This antibody was first detected by the HA test, and a peak serum titre of 1 : 32,000 occurred 226 days after inoculation. Antibody detectable by RIA did not develop until 7 days after the appearance of HA antibody, and MGD antibody did not appear until 151 days after inoculation.

Chimpanzee Yon did not demonstrate antigenaemia after inoculation, but developed HA antibody in 27, RIA antibody in 32, and MGD antibody in 151 days following inoculation. No CF antibodies were detected in either chimpanzee following initial inoculation, although when both animals were rechallenged intravenously after 357 days, with an identical concentration of initial inoculum, CF antibody appeared in both within 72 h. This suggests that this antibody is not formed after primary challenge and appears only as an anamnestic

response to antigenic restimulus. Neither chimpanzee developed clinical, enzymatic or histological evidence of hepatitis. Although it is not possible to determine whether chimpanzee Yon developed HBAb as a result of primary infection or stimulus due to initial antigenic mass, it seems likely that Xat experienced a primary subclinical infection with the virus of hepatitis B. These data indicate that the chimpanzee may be a desirable model for the experimental study of hepatitis B virus infection, and further research to establish the infection spectrum for this virus in such animals is warranted.

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Bioluminescence in the Marine Teleost, *Porichthys notatus*, and its Induction in a Non-luminous Form by *Cypridina* (Ostracod) Luciferin

THE midshipman fish, *Porichthys notatus* Girard, 1854, of wide distribution along the Pacific Coast from Alaska to Baja California, Mexico¹⁻³, is characterized by more than 700 tiny dermal photophores, arranged in rows over the body surface⁴ (Fig. 1). Except for specimens from Puget Sound, *Porichthys* photophores luminesce spontaneously only during courtship⁵. Luminescence may be evoked artificially by electrical stimulation or injection of epinephrine, norepinephrine, or amphetamine⁶⁻⁹. Strum^{10,11} reports that Puget Sound fish do not luminesce, even when injected with epinephrine, even though their photophores did not differ at the ultrastructural level from those of the luminous California variety. Professor J. A. C. Nicol (personal communication to F. I. T.) has observed that *Porichthys* collected near Friday Harbor Marine Laboratory are also non-luminous when injected with epinephrine. In luminescent *Porichthys*, light is emitted following a luciferase-catalysed oxidation of luciferin by molecular oxygen^{12,13}. Since *Porichthys* luciferin and luciferase reciprocally cross react to give light with the luciferin and luciferase of the small marine ostracod crustacean *Cypridina*^{12,13}, the possibility that *Porichthys* might obtain elements of its luminescent system from its diet¹³ has been investigated.

Specimens of *Porichthys notatus* were obtained by trawling near Santa Monica Bay, California, and at Port Orchard in Puget Sound, Washington. Eggs and early embryonic stages

were collected inter and sub-tidally near Santa Barbara, California. Since only adult males of *P. notatus*, but not *P. myriaster*, were encountered in our collections at Santa Barbara, the eggs and embryonic stages were assumed to be of *P. notatus*. *Porichthys* nests consist of 200-500 eggs (4-6 mm diameter each) firmly attached to the substrate¹⁴. Incubation requires 16-20 days, and the larvae remain attached to the substrate for a further 20-30 days. All experimental material was maintained in running, filtered seawater free from planktonic organisms larger than 30 μ m diameter. Southern California *Porichthys* were fed *Artemia* until the starvation experiments began; Puget Sound *Porichthys* were collected on July 26, 1971, and starved throughout. Luminescence capability after the various experimental treatments was assayed under light anaesthesia in tricaine methanesulphonate (Ayerst 'Finquel', 0.20 g l.⁻¹ seawater) by close subcutaneous injection of part of a gular photophore row with 10-20 μ l. of 0.001 M DL-norepinephrine in Young's teleost saline¹⁵. Two minutes after injection, luminescence was assayed in the standard way by monitoring the injected region at a distance of 6 cm with an RCA 1P21 photomultiplier (operating voltage 1,000 V) registering on an oscilloscope and chart recorder, at a sensitivity of approximately 10⁸ photons per second incident on the photomultiplier per cm deflexion of the recorder.

Extracts of ten eggs (approximately 100 mg/egg, wet weight) or of a single larva of Santa Barbara *Porichthys* were prepared by grinding the tissue in 5 ml. of 0.2 M Tris (pH 8.0) in a glass homogenizer immersed in an ice bath. Similar extracts were prepared from 0.5 g wet weight strips of dermal photophores and muscle of the Puget Sound *Porichthys*. Highly purified *Cypridina* luciferin and luciferase were prepared as described earlier^{16,17}. In the *Cypridina* reaction, the luciferase catalyses the oxidation of luciferin by molecular oxygen, yielding oxyluciferin, CO₂, and light (460 nm)¹⁸⁻²⁰. The presence of *Porichthys* luciferin or luciferase in the fish tissue extracts was determined by injecting either *Cypridina* luciferin (0.2 ml. + 1 ml. of 0.2 M Tris (pH 8.0)) or 2 ml. of *Cypridina* luciferase (1.73 $\times 10^{-5}$ mg/ml., in water) into 2 ml. of the extract, and recording initial light intensity. Controls consisted of injecting *Cypridina* luciferin and luciferase separately into the Tris buffer, and Tris buffer into the extracts. No luminescence resulted from the controls. Extracts of 10-15 adult *Artemia* prepared in 3.0 ml. of 0.2 M Tris (pH 8.0) showed no evidence of luminescent cross reactions.

The starvation experiments indicate that adult Southern California *Porichthys* do not require a continuous supply of

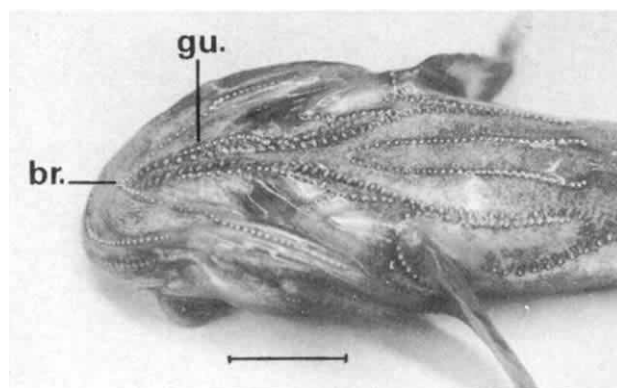


Fig. 1 Ventral view of adult *Porichthys notatus* Girard from Puget Sound. Although two species of *Porichthys*, *notatus* and *myriaster*, inhabit Southern California waters, only *P. notatus*, readily distinguished by the broadly V-shaped contour of the second (branchiostegal) photophore row, without forward projection, occurs north of Point Conception, California, and in the Puget Sound area¹⁻³. This fish (s.l. = 12.8 cm), originally non-luminescent, became luminescent in response to intraperitoneal injection of *Cypridina* luciferin. gu. = gular photophore row, br. = branchiostegal photophore row. Scale indicates 1 cm.

luminescent substrates from the diet. On days 75, 133 and 150 of starvation, four Southern California *Porichthys* (12.0–15.0 cm standard length (s.l.)) luminesced brightly in the region of a localized injection of norepinephrine. On day 133 of starvation an intraperitoneal injection of 100 μ l. of 0.001 M norepinephrine induced in all specimens a bright, whole-body photophore display lasting several hours. The ubiquity of luciferin in these Southern California fish is further illustrated by demonstration of its presence in egg yolk, embryos and pre-feeding larvae of all stages (Table 1). By contrast, luciferase was not demonstrable until just before release of the larvae from the substrate. Our finding of luciferin throughout larval tissue, but with only traces of luciferase, is similar to the situation reported for adult luminous *Porichthys*^{12,13} and indicates that Southern California *P. notatus* can luminesce before substratum release. Injection of norepinephrine into such a larva (18.2 mm s.l.) resulted in photophore luminescence. Although a very low enzyme concentration in photophore, but not muscle, extracts was detected, all ($n=6$) tests for luciferin in tissue of adult Puget Sound *Porichthys* were negative.

Six Puget Sound *Porichthys* (12.7–13.5 cm s.l.) were tested on August 9 and October 18, 1971, by norepinephrine injections. No luminescence was detectable with our photomultiplier system. On October 20, these six fish each received an intraperitoneal injection of 0.5 ml. of *Cypridina* luciferin (approximately 1 mg freshly dissolved in 0.9 ml. of Young's saline + 0.3 ml. of 0.2 M sodium phosphate buffer, pH 7.2). At 1.5 h afterwards no luminescence was detected in response to a standard norepinephrine injection. One week later, the *Cypridina*-injected Puget Sound fish were all found to have their photophores fluorescing in the green in response to near ultraviolet light and to be luminescent on norepinephrine injection. This is characteristic of the luminescent Southern California fish but not of the Puget Sound specimens. A single, non-injected, Puget Sound control fish remained non-luminescent and non-fluorescent. These conditions were maintained for 21 days until all fish except the control had died, probably of starvation. Until death, the *Cypridina*-injected Puget Sound fish remained capable of strong luminescence in response to norepinephrine. Finally, the remaining Puget Sound control fish received an injection of *Cypridina* luciferin identical to that used in the initial experiment. On the seventh day after injection it was observed to fluoresce and an injection of norepinephrine produced a short-lived luminescence that was clearly visible to the dark-adapted eye. Experiments in progress indicate that oral administration of both dried *Cypridina* and purified substrate also induce luminescence in the Puget Sound fish.

Table 1 Developmental Stage of Southern California *Porichthys*

Component present	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 22	Day 28	Artemia extract
Luciferin	+	+	+	+	+	+	+	+	—
Luciferase	—	—	—	—	—	—	—	+	—

Distribution of luciferin and luciferase in developmental stages of Southern California *Porichthys* as indicated by reciprocal cross-reactions with purified *Cypridina* luciferase and luciferin. At day 15 the embryos ruptured the egg membrane, but remained affixed to substratum. Presumably they would have detached by day 45 after attaining a length of 32–34 mm. Note that luciferin was present throughout development with luciferase minimally detectable in larvae only at day 28.

These experiments constitute the first *in vivo* demonstration of the by now well known cross reactions between luciferins and luciferases of fish and crustacea^{12,13} and indicate that the inability of Puget Sound *Porichthys* to luminesce is due to a deficiency of substrate. The fact that this failure may be overcome by both intraperitoneal and oral administration of *Cypridina* substrate suggests that this deficiency may be of a

dietary consequence. Experiments are in progress to examine the mechanisms responsible as well as the ecological implications of this phenomenon.

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Transfer of Inorganic Mercury to Milk of Goats

ALTHOUGH poisoning due to mercury in foodstuffs is well established¹, little is known about the transit of the food web by mercury compounds. Because of the importance of milk in the human diet, we have investigated how lactating animals metabolize mercury, not by administration of gram quantities of mercury as was done before^{2,3} but at environmental levels.

Three mixed-breed lactating goats, about 60 kg in weight, were maintained in collection stalls throughout the experiment and were milked, fed and watered twice daily. The daily diet was 0.5 kg of a dairy ration, 0.9 kg alfalfa hay and water *ad libitum*. Goat 32, pregnant and in late lactation, produced 1.04 kg of milk per day. Goats 169 and 170, in the first third of lactation and not pregnant, yielded 1.6 and 2.76 kg of milk per day, respectively.

Goat 32 was dosed orally with ²⁰³HgCl₂ and studied for only 9 days; goats 169 and 170 were given an oral dose and collections were made for 16 days; they were then dosed

intravenously and milk was collected for 18 days. The doses contained 60–150 μCi of $^{203}\text{HgCl}_2$ (specific activity 500 $\mu\text{Ci mg}^{-1}\text{ Hg}$). Faeces and urine were collected daily. Jugular blood samples were taken periodically and the plasma was separated immediately from the red blood cells by centrifugation. The samples of milk, urine and faeces were counted in a large-sample counter while the blood fractions were counted in a well counter equipped with a sodium iodide crystal. All counts were calculated as percentage of administered dose.

The distribution of ^{203}Hg is shown in Fig. 1; results for goat 32 are shown separately because this experiment lasted for only 9 days. After oral administration, most of the ^{203}Hg was excreted rapidly in the faeces, with a small amount appearing in the urine. The peak activity in faeces was at day 2, averaging about 50% of the administered dose, and was followed by a sharp decline. Urine activity peaked at day 3, reaching a level of 0.5% of the total dose, declined sharply and peaked again at day 9. The content of ^{203}Hg increased in milk during the first 3 days and then declined; the amount present was minimal, ranging from 10^{-7} to $10^{-5}\%$ g^{-1} . In whole blood there was an increase to a peak on day 3, followed by a decline as in the milk. Blood values ranged from 10^{-5} to $10^{-4}\%$ ml^{-1} . The data for goat 32 correlated well with those for goats 169 and 170.

Table 1 Recovery of ^{203}Hg in Urine, Faeces and Milk after Oral and Intravenous Administration of Labelled Mercuric Chloride to Lactating Goats

Goats	Oral dose			Intravenous dose			
	32	169	170	AV	169	170	AV
Faeces (%)	99.0	88.4	96.8	94.7	21.6	31.5	26.6
Urine (%)	0.4	4.5	2.8	2.9	25.7	22.3	24.0
Milk (%)	0.011	0.039	0.006	0.019	1.09	0.77	0.9

Data are expressed as percentage of the administered dose.

The recovery of an average of 95% of the radiomercury in the faeces after oral dosing (Table 1) implies very poor absorption, consistent with evidence for other species^{2,3}. The total recovery in urine varied from 0.4 to 4.5%, while only 0.006 to 0.039% was recovered in the milk.

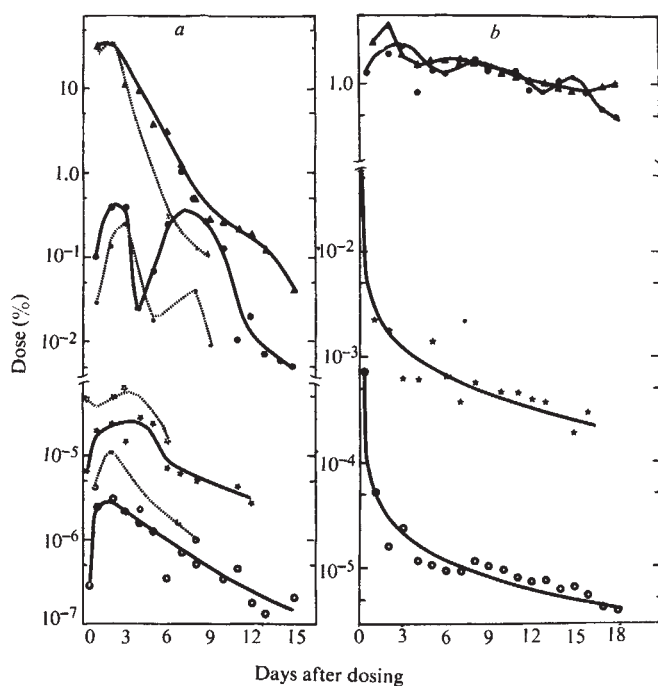


Fig. 1 The patterns of distribution of radiomercury, given at HgCl_2 , in milk, urine, faeces and blood after (a) oral and (b) intravenous dosing. $\blacktriangle$, Faeces; $\bullet$, urine; $\circ$, milk g^{-1} ; $\star$, blood ml^{-1} ; $\cdots$, goat 32; —, goats 169 and 170.

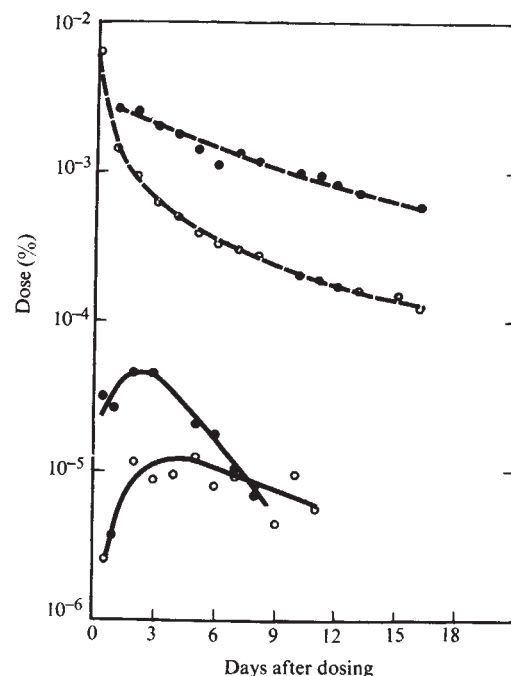


Fig. 2 The distribution of ^{203}Hg in plasma and red blood cells of goats after oral (—) and intravenous (---) dosing. $\bullet$, Red blood cells; $\circ$, plasma.

After the oral studies, goats 169 and 170 were given intravenous $^{203}\text{HgCl}_2$; the loss patterns for urine, faeces and milk are shown in Fig. 1; the totals are in Table 1. In 18 days about 25% was excreted in the urine and in the faeces to give a total of about 50%. The total recovery from milk was about 0.9%, about 2.5 times higher than expected from the oral dose data obtained with these goats. Nine days after intravenous dosing there was about a 100-fold difference between blood and milk contents of ^{203}Hg , compared with a ten-fold difference when HgCl_2 was given orally.

The pattern of distribution of ^{203}Hg in blood after intravenous dosing differs from that after an oral dose (Fig. 2). Ten minutes after an intravenous dose the red blood cells contained no detectable ^{203}Hg but after 24 h and later they contained more radiomercury than an equal volume of plasma. The loss of mercury from the blood cells could be described by a simple exponential function with a $t_{1/2}$ of 5.5 days. Loss from the plasma was multiexponential with a $t_{1/2}$ of 0.5 days for the first component and 7.2 days for the second. After oral dosing the red blood cells contained more radiomercury than the plasma at all sampling periods up to 7 days; after that the ^{203}Hg in the plasma exceeded that of the red blood cells. This crossover was apparently due to a greater rate of loss from the cells. Analysis of haemolysed red blood cells showed that ^{203}Hg was associated with the cellular contents and not with membranes.

The differing mercury distribution in blood and loss of mercury after oral and intravenous doses suggest that different forms of mercury may be being compared. The bacterial flora of the rumen, intestine and caecum could transform part of the inorganic mercury given orally into an organically bound form. This could produce a pattern in blood that would be unlike that which results when HgCl_2 is given intravenously, so that a greater proportion of the absorbed mercury would be transferred to milk. The formation of methylmercury from dietary inorganic mercury has been observed in some animals^{4,5} and membranes have been noted to block the transfer of inorganic mercury while allowing some forms of organic mercury to pass^{6,7}.

These data indicate that inorganic mercury is poorly absorbed from the digestive tract and only a small fraction of that absorbed is transferred to milk. In terms of the food web, the lactating animal acts to reduce the transfer of environmental mercury to man.

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Enhancement of Bacterial Methane Oxidation by Clay Minerals

METHANE is abundantly liberated by bacteria from the sediments of rivers and lakes¹. If methane formation exceeds its solubility in water it is released into the atmosphere². Several species of bacteria oxidize methane as an energy source, converting it into cellular materials or carbon dioxide gas³. Accumulation of carbon derived from methane is involved in the ageing of lakes because it causes some carbon to be recycled in the aquatic ecosystem rather than lost as gas. How much methane carbon is retained in the ecosystem and how much escapes into the atmosphere depends on conditions in the water. This report describes enhancement of bacterial methane utilization by clay mineral particles in a simulated aquatic ecosystem.

Bacterial inocula were taken from a mixed culture, isolated from Lake Erie, that was rich in methane oxidizing bacteria and had reproducible and predictable growth patterns. One ml. of this culture, chiefly Gram negative cocci and rods, was used as required to seed experimental vessels.

The common types of clay minerals tested and their respective cation exchange capacities (mequiv/100 g) were: kaolinite (9.7), illite (24.6), vermiculite (70.6) and bentonite (74.5). Organic material was removed from the clays by extraction with 0.5 N NaOH (ref. 4).

Experiments were performed in 74 ml. serum bottles fitted with rubber stoppers. Necessary additions were made to the bottles, then mineral salts medium was added to bring the volume to 25 ml. The bottles were filled with a methane: air mixture, and methane utilization and carbon dioxide production were monitored by gas chromatography.

When quantities of kaolinite (0.004% to 4.0%) were tested for their effect on methane uptake by bacteria, all concentrations enhanced methane oxidation. There was a decrease in the lag period before onset of methane oxidation, total methane uptake increased, and the rate of methane oxidation increased. The 4.0% kaolinite suspension produced maximum stimulation.

There were no significant differences in methane oxidation stimulation between the four clay minerals, which indicates that cation exchange capacity is not responsible for this enhancement. A surface phenomenon is implied because smaller clay

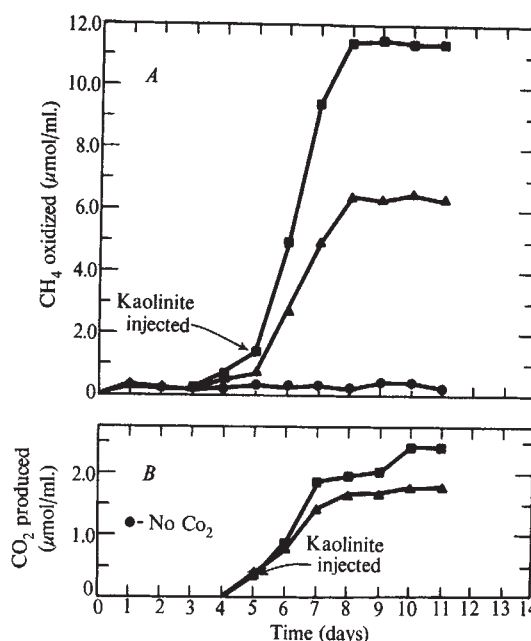


Fig. 1 Curves showing (A) amount of methane oxidized and (B) amount of CO₂ produced by a mixed bacterial population versus time of incubation when kaolinite (4%) was added to actively growing cells. ●, Control sterile medium. ▲, Control medium plus cells. ■, Control medium plus cells to which kaolinite was added on the fifth day.

particles (<2 μm) with a greater surface area/unit volume stimulated methane uptake more than did larger particles (>2 μm). The enhancement probably involves one of the following: (i) adsorption of a repressor or inhibitor, (ii) increased availability of nutrients, or (iii) provision of some unknown stimulating factor by the clays.

In nature it is probable that a continuously growing population of methane oxidizing bacteria is exposed to concentrations of clay particles which are varied by dispersal of bottom sediments into the water columns and surface runoff by wind and wave action. When kaolinite was injected into an actively growing culture of methane oxidizing bacteria the rate of utilization of methane was doubled and the total quantity taken up increased (Fig. 1).

We conclude that clay mineral particles enhance methane consumption by methane oxidizing bacteria. The increased utilization of methane in an aquatic ecosystem would increase methane carbon retention in water and thereby accelerate total carbon accumulation. The significance of this effect is emphasized by consistently high *in situ* measurements of methane production in Lake Erie sediments (1.71 cm³ CH₄/min/m²)¹. Thus microbial fixation of methane can be an important contributor to the accumulation of organic material in lakes which may play a key role in lake eutrophication⁵. One might reduce methane carbon retention, and thereby eutrophication, by reducing runoff of clay minerals into rivers and lakes.

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BOOK REVIEWS

Anatomy of Innovation

Wealth from Knowledge: Studies of Innovation in Industry. By J. Langrish, M. Gibbons, W. G. Evans, and F. R. Jevons. Pp. xiii+477. (Macmillan: London, January 1972.) £4.95.

Success and Failure in Industrial Innovation: Report on Project Sappho by the Science Policy Research Unit, University of Sussex. Pp. 36. (Centre for the Study of Industrial Innovation, 162 Regent Street, London W1, February 1972.) £0.75.

THE troubled air is thick with rival terminologies: pure, applied, strategic, tactical research jostle for a place in the sun of the master's approval. It is perhaps just as well that the green paper did not try to grapple with the intricacies of the concept of industrial innovation, a concept which is not equivalent to invention, or R and D, or commercial profitability, or an efficient productive process, yet incorporates all of these, and more. Neither of the admirable volumes under review is oppressed by lexicographic accidie, but the reader is left in no doubt that he is learning something valuable about a clearly identifiable and unmistakably important process.

Both books are based on an analysis in depth of a number of case-histories. *Wealth from Knowledge* concerns itself with eighty-four innovations cited in the sixty-six Queen's Awards to British industry made in 1966 and 1967 specifically for prowess in technological innovation. *Project Sappho* is based on a statistical correlation analysis of a number of features associated with twenty-nine pairs of innovations in the chemical and scientific instrument industries; each pair, critically selected from a larger number of starters and closely matched as to theme, consisted of one "successful" and one "unsuccessful" innovation. While both studies derive as much quantitative information from their case histories as possible (for example, *Wealth from Knowledge* establishes significant distinctions between firms making "major" and "minor" innovations), the statistical methods used by the Sappho

team are particularly subtle: the aim here is to correlate success with specific features of the successful firms. This technique made it possible to pinpoint significant differences in the criteria for success as between the chemical and scientific instrument industries. Both sets of case-histories are based on extensive interviews as well as correspondence and published material: the conclusion in *Wealth from Knowledge* that information transfer is most effective "on the hoof" is well exemplified by the staff who assembled the case-histories.

Wealth from Knowledge incorporates a background discussion and an extended set of conclusions followed by quantitative tabulations of various characteristics of the innovations; the remainder of the book consists of the detailed case-histories. There are extensive bibliographies. *Project Sappho* is itself a concise summary of a much longer account which can be obtained from the Science Policy Research Unit at Sussex University; the summary contains a fairly detailed account of the methodology and statistical methods, the actual results derived from statistical analysis, and then relates these to a large range of hypotheses concerning industrial features that might be expected to foster innovation. The summary, unlike the full account, incorporates only a modest bibliography.

Although the two sets of case-histories were selected on quite different criteria and the quantitative methods used were also quite different, there is an impressive measure of accord between the two sets of conclusions, and in particular both studies agree in slaughtering some of the same sacred cows. Nevertheless there are also one or two important contradictions between the two studies. They should certainly be read in conjunction.

In outline, *Wealth from Knowledge* pinpoints the following as the factors that enable award-winning firms to succeed: (1) the presence of an outstanding person—a product champion—in a position of authority; (2) the presence of a person with exceptional technical knowledge or skill; (3) clear

identification of a need followed by a determined attempt to meet it; (4) the recognition of the value of an earlier discovery, often made elsewhere; (5) availability of money and manpower; (6) help from government sources. *Project Sappho* puts its strongest emphasis on the importance of recognition of user needs and (almost a corollary, it would seem) on conscientious attention to user education once the instrument or process is available for sale. The third conclusion is that successful firms do their R and D more efficiently than the failures, but (surprisingly perhaps) speed of R and D is not important. (This is inconsistent with the emphasis of a government report on technological innovation, published some years ago, which placed great emphasis on the importance of minimizing lead-times.) *Project Sappho* agrees about the vital role of responsible individuals, but places more emphasis than *Wealth from Knowledge* on the importance of having product champions in positions of authority. Finally, successful innovators make more effective use of outside technology and also of scientific advice, though they rarely contract out their R and D. This conclusion links with the finding of *Wealth from Knowledge* that technology transfer "on the hoof" is more effective than transfer through the printed word: altogether, the "scientific literature" is put severely in its place, which seems to be mostly as a means of communication between pure scientists. Nevertheless, one of the case histories quoted in *Wealth from Knowledge*, the development by ICI of the Procion dyes, arose from one scientist's inveterate scanning of the literature.

The very strong emphasis in both books on user needs leads on to an intriguing question: is need-pull always a more important component of successful innovation than discovery-push? The authors of *Wealth from Knowledge* address themselves in depth to this important question: it is one which lies at the root of the current debates about the customer/contractor principle and has been much discussed, especially by the champions of the MRC and ARC. The supremacy of

need-pull is implicit in Lord Rothschild's axiom. In spite of their third conclusion the authors observe that need-pull and discovery-push were often extricably mixed in their case histories. Mirrlees's identification of a need for smaller Diesel engines, J & S Pumps' identification of a need for sealed corrosion-proof pumps, Martin-Baker's decision to design an effective aircraft ejector seat, were all clear instances of need-pull. But the development by Thorium Ltd of cheaper ways of separating the rare earths was a classical instance of a programme based on the decision to exploit a new technique, solvent-extraction. The firm did not engage in market research or in laboratory research on possible application and trusted to future scientific developments in the world at large to create a market by the time they were ready to supply it: they have been justified by events, but this particular success story is not consistent with green paper principles. The development of translucent china by Doulton and of synthetic penicillin derivatives by Beecham are examples where need-pull and discovery-push were inextricably intermingled.

Both books examine a number of factors which some commentators have considered important, such as size of firm, organizational structure or management techniques, use of large numbers of qualified scientists and engineers. The ones listed here do not seem to be important one way or the other. Thus, one successful firm (J & S Pumps) took great pains to remain small, others are among the largest. Some used patent cover extensively, J & S Pumps did not find this worth the expense. Some depended on external contracts, whereas Sir James Martin of Martin-Baker avoided R and D contracts on principle so as to preserve his company's freedom to use its own developments as it wished. (A worthwhile investigation is waiting to be done on the correlation between reliance on R and D contracts, either in-house or external, and commercial success in science-based firms.)

One sad but inescapable conclusion from the work of *Wealth from Knowledge* is that few successful innovations stem from university research: of the case histories in *Wealth from Knowledge* only the stereoscan electron microscope (also analysed by *Project Sappho*) clearly emerged from an academic laboratory, while Beecham's development did depend crucially on academic consultants. The conclusions of *Project Sappho* also place considerable emphasis on the importance of making use of the best scientific advice, which often comes from academic sources. On a statistical view, however, *Wealth from Knowledge*

confirms that technology grows out of technology, and the world of pure science primarily provides skilled manpower and novel techniques of investigation, apart from the vital consultancy services.

These two studies have established new standards for the effective investigation of the infinitely subtle process of industrial innovation and any future investigations will inescapably build on the foundations, methodological and factual, established by these pioneers.

R. W. CAHN

Uses of NMR

The Nuclear Overhauser Effect. By Joseph H. Noggle and Roger E. Schirmer. Pp. xi+259. (Academic: New York and London, October 1971.) £6.75.

Magnetic Resonances in Biological Research. Edited by C. Franconi. Pp. xii+408. (Gordon and Breach: New York and London, December 1971.) £10.20.

FROM studying the change in the integrated intensity of a nuclear spin when another nuclear spin in the system is saturated by irradiating at its resonance frequency, it is possible to comment on the relaxation mechanisms of the nuclei, to study inter- and intra-molecular exchange processes in solution, to make spectral assignments and in favourable cases to obtain molecular configurational and conformational information. Such a potentially useful technique deserved the detailed discussion which Drs Noggle and Schirmer have provided in their timely book on the subject. The theoretical basis of the nuclear Overhauser effect is well presented and the authors have chosen to include the basic relaxation theory necessary to the understanding of method. They deal in a systematic manner with these effects in multispin systems and show how in favourable cases in rigid molecules it is possible to obtain internuclear distances. A critical review of most of the applications reported is included and it is obvious that many of the reported experiments have not been conducted in the rigorous conditions recommended by the authors in a useful chapter on experimental methods.

The exciting possibility of obtaining conformational information in non-rigid molecules by the nuclear Overhauser method was not fully developed in the book and clearly this is an area which requires more attention. The problem of interpreting experiments when there are several conformations present in dynamic equilibrium is con-

sidered briefly. It is obvious that the problem of deciding relative magnitudes of the relaxation rates and exchange rates is important in selecting the appropriate theory to evaluate the function describing the effect of having a distribution of the molecules among the possible conformations.

In general, the presentation of the subject-matter is clear, although the separation of the molecular structures from the remainder of the text in chapter 8 is somewhat irritating, and on page 170 (Fig. 8.2) the lettering on the methyl groups of the citral structures is different from that shown on page 208.

This interesting book can be recommended not only to the nuclear magnetic resonance worker contemplating using this technique, but to anyone who has occasion to use nuclear magnetic resonance techniques for molecular structure determinations.

Magnetic Resonances in Biological Research comprises a collection of thirty-eight research papers presented at the Third International Conference on Magnetic Resonances in Biological Research held in Cagliari, Italy, in 1969. As such it suffers from the general problems associated with reporting the proceedings of a conference, namely, that many important areas are inadequately covered and that there is often a lack of cohesion between the many disconnected papers. This is made all the more obvious by the fact that some of the more important papers are presented in an abbreviated form without tables or figures. One of the potentially useful aspects of a book of this type is that it presents an opportunity to report fruitful discussions following the papers at the meeting. Unfortunately little or no discussion is reported in this book.

More than two-thirds of the articles are concerned with electromagnetic spin resonance (ESR) studies and the review of ESR studies on haemoglobin and its derivatives by D. J. E. Ingram is one of the articles which is ideally suited to presentation in textbook form. There are several papers on haemoglobin which should be of interest to those working in this area. Useful surveys of the ESR spectra of low spin (Blumberg and Peisach) and high spin (Kotani and Watari) ferric ions in proteins are presented. Shulman and his co-workers used nuclear magnetic resonance (NMR) to obtain information on the role of the haem group during the cooperative oxygenation of haemoglobin.

There are several interesting papers on protein-ligand interactions. Nemethy and Laiken have considered the problems encountered in the calculation of protein conformations and the forces involved in protein-ligand interactions. Jardetzky and Markley report NMR studies on selectively deuterated staphy-

lococcal nuclease to follow conformational changes on denaturation and the effects of binding small molecules to the protein. Unfortunately, their paper consists simply of a summary of their results, and unless the reader is already familiar with their elegant deuteration technique the impact of this important paper will be lost. Raftery and Parsons show how one can study inhibitor and substrate associations with lysozyme by measuring the chemical shift changes which occur when the small molecules bind to the protein.

A novel method for obtaining conformational information for fibrous proteins is described by Chapman and co-workers. By replacing the exchangeable protons with deuterium in molecules such as collagen and observing the doublet splittings in the deuterium spectrum when the fibre axis is coincident with the applied magnetic field they were able to interpret the splittings in terms of the conformation of the backbone.

Because much of the work described at this meeting has also been presented in more detail in the scientific literature one might question the value of such a text. But because of the lack of any textbooks which cover adequately the applications of magnetic resonance in biology the existence of a volume of collected papers is not without value.

There is no reported discussion of the papers, and the book therefore fails to communicate any of the exchanges of ideas which surely take place at such a meeting and which would greatly have enhanced its usefulness. J. FEENEY

Carnot Père

Lazare Carnot, Savant. By Charles Coulston Gillispie. (A Monograph treating Carnot's Scientific Work, with facsimile reproduction of his writings on mechanics and on the calculus, and an essay concerning the latter by A. P. Youschkevitch.) Pp. xi+359. (Princeton University: Princeton, New Jersey; Oxford University: London, October 1971.) £8.25.

LAZARE CARNOT is known to the general public as the "Organizer of Victory" of the French Revolutionary armies, and to physicists as the father of his elder son Sadi. He was, however, also a *savant* in his own right, and in this monograph Professor Gillispie gives us an account of his work in mechanics and, to a lesser degree, in geometry and the infinitesimal calculus.

His mechanics were soon overtaken by those of the mathematical giants of the late eighteenth and early nineteenth centuries, many of whom were his own countrymen—Lagrange, Laplace, Pois-

son, Coriolis and Navier. What is still of great interest in his work is the extent to which it influenced Sadi's pamphlet of 1824, *Réflexions sur la Puissance Motrice du Feu, et sur les Machines Propres à Développer cette Puissance*. This work was once thought to have been almost an isolated phenomenon in the history of thermodynamics, without parentage and, for many years after its publication, without issue also. It is now becoming clear that its apparent isolation is a myth. Thus it has been accepted for some years that Sadi's ideas on the flow of heat (how the old "caloric" language still pervades our thought!) from a higher to a lower temperature were influenced by his father's on the flow of water in the operation of hydraulic machinery. More recently Fox has emphasized what Sadi owed to Watt and the practical engineers. Now Gillispie argues persuasively that an even stronger influence on Sadi's thought was his father's concept of "geometrical motions". These are essentially what we should now call virtual displacements, but embody also the important idea of reversibility. To get the best performance out of machinery we must avoid all shocks or percussions between surfaces moving at different speeds, just as we must avoid all transfer of heat across non-zero intervals of temperature if we are to get the best out of a heat engine.

The book includes facsimiles of three of Carnot's manuscripts, discovered by the author in the course of his research; two early memoirs on the theory of machines, and the earliest version of his work on the calculus. The last is introduced by an essay by Professor Youschkevitch of Moscow. The manuscripts are clearly all fair copies and so throw no light on the way Carnot's thoughts were running. Since even the most elegant French handwriting of the eighteenth century is tiring to read when printed in black on a grey background, it is hard to understand why these memoirs were not set in letterpress. However, there is so much to be grateful for in this handsome volume that perhaps we should not cavil at three fashionable facsimiles.

J. S. ROWLINSON

Fat Cells

The Human Adipose Cell: a Model for Errors in Metabolic Regulation. By David J. Galton. Pp. viii+220. (Butterworth: London, October 1971.) £4.

THIS slim volume on the human adipose cell is aimed primarily at physicians and postgraduate medical students whose knowledge of adipose tissue metabolism

may be non-existent or in need of a brush up. The author has explained in the preface that this is a book which covers the field extensively and superficially rather than in depth and is therefore unlikely to be suitable for research workers. The field of adipose cell physiology and biochemistry has developed explosively following the pioneering studies of Wertheimer. It is important in relation to nutrition, diabetes, obesity and atherosclerosis, and the adipose cell has illuminated studies of fat and carbohydrate metabolism, metabolic regulation and hormone action. There are at least three recent books with papers by distinguished workers in the field which review various growing points in the subject. Adipose tissue is not usually well covered in physiology or biochemistry texts and since much of the knowledge of this important tissue is very recent there may well be many with little knowledge of it. There is thus a need for an elementary text both to convey knowledge about this cell and to put across the dogma and jargon of eukaryote metabolic regulation to excite the growth of clinical investigation. This book could succeed but it has three shortcomings. In many instances I felt that the reader would be left in the air with no guidance as to where to go for more detailed knowledge of a particular topic that might be of interest. The book contains a number of irritating errors, some of which are unfortunate and will not be obvious to those whom it is designed to instruct. The bibliography is given in relation to numbers in the text and is not arranged in alphabetical order of authors. This is a pity because the book contains references to much scattered work on human adipose cells which has been poorly reviewed elsewhere. Any other presentation of the bibliography could have made the book a useful work of reference on human fat cells.

The book can be recommended as a modest account of metabolic regulation in animals as exemplified by the fat cell and as a source of reference for work on human adipose tissue metabolism in which field the author is known as an original contributor.

P. J. RANDLE

Trematodes in Molluscs

Flukes and Snails. By C. A. Wright. (No. 4.) Pp. 168+16 plates. (George Allen and Unwin: London, November 1971.) £3.25 cloth; £1.75 paper.

THE book concentrates on the larval stages of the digenetic trematodes and their relationships with their molluscan

hosts and attempts to redress the usual bias towards the adult worms and their vertebrate hosts. It opens with an account of the flukes which is largely concerned with phylogeny. The conventional derivation of the Digenea from turbellarian ancestors is questioned and it is suggested that they may have evolved with the Mesozoa and the Aspidogastrea, and have no relationship with the rest of the Platyhelminthes. The six living classes of mollusc are briefly described and attention is focused on tissues and processes which are important in their role as hosts. Three chapters deal with the successive stages in the life-cycles of flukes from eggs to cercariae, and with the influence of environmental conditions on successful development and transmission. The chapter on host-parasite relationships covers the obvious topics such as tissue responses and immunity and goes on to describe the fascinating situations which arise with multiple infections. The book ends with an account of the taxonomic difficulties which arise from the inherent variability of both flukes and snails and of the application of biochemical techniques to help solve the problems.

The author has assembled a remarkable number of facts from the literature and has constructed a useful picture of what is known and how much remains to be investigated. He has tried to avoid undue emphasis on schistosomes and liver-flukes to achieve a balanced account and to draw attention to less well-publicized studies. This seems to have led to the omission of most available observations on the quantitative aspects of transmission. Topics such as the survival and infectivity of cercariae and the epidemiological effects of dams and irrigation schemes are not discussed. The book fills a gap in the existing literature and can be read fairly quickly. It should prove a useful introduction for those who are not already involved with these animals. Indeed, few readers can fail to learn something new but some will find it frustrating that only 129 references are cited.

A. D. BERRIE

Maintaining Polymers

Polymer Stabilization. Edited by W. Lincoln Hawkins. Pp. viii+452. (Wiley: New York and London, February 1972.) £11.75.

THERE seems to be a growing tendency for scientific books to be written by groups of authors; there are obvious attractions for the contributors but the advantages for the readers may be accompanied by some rather serious dis-

advantages. The readers can expect that the various contributors are real experts in their fields and that they can write authoritatively. On the other hand, there may be considerable differences between the styles adopted by the authors; in some cases, there is considerable overlap between chapters, and yet an absence of adequate cross-references. Strong and effective editing is essential for a book of this type to be really successful. Dr Hawkins has obviously done a great deal to ensure that the various chapters of *Polymer Stabilization* fit together properly. His task may have been eased by the fact that he wrote the first and last chapters but it is significant also that colleagues of his at the Bell Telephone Laboratories wrote four of the other eight chapters.

The prevention of physical and chemical changes in polymers during storage, processing and use is obviously a matter of great importance; without it, there would be serious and deleterious changes in the properties of the materials and failures would occur far too readily. In many cases, profound effects can result from quite small chemical changes; thus, in a typical polymer, breakage of only one valency bond in 20,000 may lead to a halving of average molecular weight. Dr Hawkins and his team have acted upon the sound principle that any effective approach to the problem of stabilization must be based on a proper understanding of the mechanisms of the various changes which can lead to deterioration. There is little sign of this book degenerating into a catalogue of additives and their applications; instead, in the main, it presents a fundamental analysis of the problem and possible solutions.

Two of the longest chapters concern reactions of polymers with oxygen at quite low temperatures and another deals with effects of ozone; other chemical reagents are dealt with more briefly. One chapter is devoted to the effects of high temperatures on systems essentially free from oxygen; its prime significance is therefore in connexion with the processing of polymers although degradation in these conditions has received much academic attention. Another chapter deals with the combustion of polymers and methods employed to make them less flammable. A long chapter on the effects of high energy radiations and on protective agents includes very little on biological polymers. There is a chapter concerned with biological stability of polymeric materials. Dr Hawkins himself has contributed a useful introductory chapter and a final chapter dealing with testing procedures.

The book can be strongly recommended but it is perhaps unfortunate that it contains nothing about the disposal of waste polymers. It would have

been quite appropriate to have considered this subject which is, after all, closely connected with mechanisms of degradation; for example, the chapter on combustion might have been extended to include discussion of the practical difficulties of incinerating polymers.

J. C. BEVINGTON

Complications of Spin

Spin and Isospin in Particle Physics. By Peter Carruthers. Pp. ix+258. (Gordon and Breach: New York and London, November 1971.) \$19.50; £8.10.

PROFESSOR CARRUTHERS is well known to graduate students in theoretical particle physics for his excellent little monograph on SU(3) symmetry. His new offering on spin and isospin is, therefore, something of a disappointment.

The book divides naturally into three parts: the first deals with the homogeneous and inhomogeneous Lorentz groups; the second with the discrete transformations and isospin; the final one with some applications of the previous two parts to three and four point functions of physical interest. It is the only book known to me that includes more recent developments such as Weinberg's approach to high spin fields. It fills a gap left by traditional volumes on field theory which never find the space to discuss spins beyond 1. This book not only deals with arbitrary spin fields but also discusses their application to phenomenology in a way which does not depend on perturbation theory.

The author's style throughout is sparse and dry rather in the manner of lecture notes. This is no introduction to the subject: a first year graduate student is served far better by the numerous excellent texts on particle physics that have appeared in recent years such as those by Gasiorowicz or Martin and Spearman, to mention only two. In general, the book reads more like a research paper than a book written for graduate students.

In spite of these drawbacks, this volume is a valuable addition to the literature on spin and its complications. It is a useful reference book and is adequate as a brief guide through the confusing literature on high spin by assembling the bare essentials in a compact form. For those graduate students seeking an introduction to the subject it will not be as useful as one might have hoped, and at £8.10 for 258 pages of terse writing it will have nothing like the appeal of its predecessor on SU(3).

P. G. WILLIAMS

CORRESPONDENCE

Katchalsky Memorial Fund

SIR,—The senseless murder of Professor Aharon Katzir-Katchalsky at Lod Airport has deeply shocked the world. Wide circles, both scientific and non-scientific, have conveyed their outrage and sorrow to the people of Israel and the Weizmann Institute of Science. We have received a flood of letters and cables all expressing a profound sense of loss at the tragic death of a scientist whose personality and impact were, literally, unique.

Many of these letters and cables have indicated a desire to participate in the creation of a memorial worthy of Aharon Katzir. We are all most deeply moved by this response. While we have not yet been able to make any detailed plans for a suitable way of commemorating Professor Katzir, we are clear on one point: the wish, at one and the same time, to perpetuate his memory on this campus and to further those fields of endeavour that were closest to his heart.

In order to make possible some kind of coordinated action, an international committee is being formed, and a Katzir Memorial Fund has been established at the Weizmann Institute of Science.

Ideas and offers of help will be gratefully received.

Yours faithfully,

I. DOSTROVSKY

*Vice-President,
Weizmann Institute of Science,
Rehovot*

Katchalsky Memorial Symposium

SIR,—Many scientists from around the world have had the privilege of knowing Professor Aharon Katchalsky, his brilliant scientific work, his extraordinary faculty to communicate science, and his warm personality. Those who have worked with him, were his students, or merely heard him lecture will carry equally the intellectual excitement which Aharon Katchalsky could invoke.

On May 30, 1972, Professor Aharon Katchalsky, returning to Israel from one of his frequent trips abroad devoted to international scientific cooperation, was gunned down at the Tel Aviv airport along with Puerto Rican pilgrims and other innocent bystanders in a vicious and senseless terrorist attack.

We have requested that a suitable

Memoriam symposium be held at the forthcoming Fourth International Congress of Biophysics sponsored by the IUPAB and due to begin in Moscow on August 7. Professor Katchalsky was a past president of this organization and an honorary vice president at the time of his demise. Because so many of his friends, students and scientific colleagues will gather in Moscow for this meeting, we feel that it is a unique opportunity to honour Professor Katchalsky's scientific and intellectual accomplishments, and would be an especially significant and fitting tribute to this outstanding international scientist. Indeed, his leadership and example in international scientific co-operation have played a major part in making such international meetings possible.

Realizing the difficulty of making late changes in a carefully prepared programme, we hope the organizing committee of the Fourth International Congress of Biophysics will see fit so to honour Professor Katchalsky with a memorial symposium.

We ask our colleagues throughout the international scientific community to express the appropriateness of such a memorial symposium, and appreciation to the organizing committee for their efforts in arranging the tribute.

Yours faithfully,

JAMES F. DANIELLI
JOHN C. ECCLES
ROBERT REIN
HAROLD SCHERAGA
ROBERT A. SPANGLER
V. S. VAIDHYANATHAN

*State University of New York,
at Buffalo,
Amherst,
New York 14226*

Science in India

SIR,—The suicide by Dr V. H. Shah (*Nature*, May 19, 1972) is not the only case in New Delhi. In the late 1950s Dr Joseph also committed suicide in similar circumstances. Many young scientists in India run into such problems but consider it wise to emigrate.

Scientific methods require proper management to produce results. The partnership between management on one hand and science on the other is an absolute necessity if improvement is to be expected in developing countries. Dr Homi Bhabha, the late Indian nuclear physicist, was probably referring to this

problem, I believe, when he said in his last speech, "It is my personal view . . . that the general absence of the proper administration for science is a bigger obstacle to the rapid growth of science and technology than paucity of scientists and technologists because we are less effective through the lack of right type of administrative support."

Will the Government of India do something about the problem? It remains a big question.

Yours faithfully,

G. R. SAINI

*Research Station, PO Box 280,
Fredericton, NB, Canada*

Science Criticism

SIR,—For a long time it has been the accepted prerogative of every newspaper to criticize the policies and attitudes of any government; any art medium has a following of eager critics willing to distinguish between the good, the bad and the ugly; and what has science got? Nothing.

For some reason science is sacred. It has always been considered bad form to criticize scientific papers without, at least, providing an alternative argument on the same subject. But why is this so? Presumably the quality of scientific work is, unlike everything else, self-evident. Or perhaps the pride of the average scientist is more tender than that of, for example, the average film producer. Or perhaps scientists are simply above criticism. None of these explanations is really satisfactory. And when one discovers how much poor work is being done in the name of science and being published without a whisper of complaint from anyone, one goes on wondering why this is so.

It seems to me that a reasonable case could be made for devoting some energy to the serious art of scientific criticism.

Yours faithfully,

ANTON ZIOLKOWSKI

*Seismic Discrimination Group,
Massachusetts Institute of Technology,
42 Carleton Street,
Cambridge,
Massachusetts 02142*

Space Tribology

SIR,—In a report entitled "Base for Britain" published on May 26 (*Nature*, 237, 189; 1972), it was stated that the contract for the European Space Tri-

bology Laboratory at Risley had been awarded for four years "because ESRO's financial future after 1975 is uncertain". The initial period of financing was determined by budget release policy and not by any uncertainty as to the availability of funds after 1975.

Last December the ESRO Council unanimously approved not only the Organization's 1972-74 budget but also a substantially increased provisional total level of resources of \$110 million a year for the three-year period 1975-77. The Council authorized ESRO to spend not less than \$70 million a year on application satellites alone during the period 1974-80.

Yours faithfully,

P. M. BROWN

European Space Research Organization,
114 Avenue Charles-de-Gaulle (92),
Neuilly-sur-Seine

Knuckle Walking

SIR,—The delightful ambiguities that can be produced by simple combinations of English (British/American) words and phrases are never-ending. A case in point is found in the recent correspondence (*Nature*, **236**, 472; 1972) from Mr Tiratsoo referring to the knuckle walking article earlier (*Nature*, **236**, 34; 1972). Mr Tiratsoo, obviously reared in the realm of British football, confuses the authors' expression "... football linemen" ... as meaning the official who in British-style football prances about (handsomely attired!), waving his little flag and (sometimes?) clutching a stop-watch. What the authors meant, and what must be obvious to an American reader, is the American football lineman—a tackle, guard, or centre who assumes

a tripodal stance, cleated feet dug in, one hand in a semi-fist, crouched and ready for the mayhem of scrimmage. In this stance the fingers are knuckled under, pressing into the turf (artificial?) before the snap of the ball from centre. The fingers are knuckled under to avoid the extremely painful injury that can result from having the opposing lineman (275 lb and up) step on outspread fingers. Most American football linemen bandage the hands so that only the knuckle stance is possible. Phrases, like landscapes, are always comprehended with respect to the point-of-view!

Yours faithfully,

C. E. LONG

Nuclear Effects Laboratory,
Kirtland AFB,
New Mexico

Obituary

Professor J. S. Griffith

THE death of Professor John Stanley Griffith at Cambridge on April 23, 1972, at the early age of 43, has brought to an end a remarkably varied career. The fact that his father was a distinguished bacteriologist and his mother a graduate in mathematics and physics may have encouraged him to think widely and discard traditional limitations in his research. In 1948 at Trinity College, Cambridge, he obtained a brilliant first-class in the mathematical tripos. This led to a Rouse Ball studentship, also at Trinity, and the opportunity to take Parts I and II of the biochemistry tripos. His early research was with Sir John Lennard-Jones at Cambridge, and then with Professor C. A. Coulson at Oxford. During this time he was a Senior Demy at Magdalen College. A year of national service at the Admiralty Research Laboratory studying the formation of droplets and the magnetic properties of certain materials was followed by the Stokes Studentship at Cambridge, the Berry-Ramsey Fellowship in Mathematics at King's College, Cambridge, and a series of chairs, first in chemistry at the University of Pennsylvania, then in mathematics at the Manchester College of Science and Technology, and at Bedford College, London. This finally led to a chair of chemistry at Bloomington, Indiana, and—for the last few months of his life—membership of the Institute of Immunology at Basle.

This varied professional career showed itself in his research. His early

work was in theoretical chemistry, where he made useful contributions to the newly-developed free-electron model for conjugated molecules, and to the spatial correlation of electrons in atoms. But by 1954 he had begun to turn his attention towards inorganic chemistry. The astonishing upsurge of interest in ligand-field theory which occurred in the late 1950s was in no small measure due to John Griffith and Leslie Orgel. Orgel concentrated on the more pictorial and descriptive aspects, Griffith on the basic principles. His immense book *The Theory of Transition Metal Ions* (1961) was a magnificent piece of work, and for the first time really established the subject on a solid theoretical basis. In it he rationalized the magnetic and optical properties of molecular complexes, taking proper account of temperature effects and spin-orbit interaction. Ten years later it still remains the definitive work in this field. This phase of his life was rounded off by a second book, *The Irreducible Tensor Method for Molecular Symmetry Groups* (1962).

By this time he was getting deeply interested in biological matters. The connecting link was the magnetic property of the combination of oxygen with haemoglobin. How could ligand-field theory account for the newly observed electron-spin resonance spectrum of ferric haem in myoglobin, with peaks at $g=6$? What was the electronic structure of the bond between oxygen and ferrous ion? One fact which had to be explained was that oxyhaemoglobin

was diamagnetic, while oxygen itself is paramagnetic. Griffith showed that there were two possible answers. The one, which he preferred, was completely original, and postulated a symmetrical structure, with the iron-oxygen bond perpendicular to the oxygen-oxygen bond. The other was a reinterpretation of Pauling's bent bond structure, translated into molecular-orbital terms. Griffith's interpretation of the electron-spin resonance spectra was immediately accepted, and proved most valuable. His symmetrical model for oxygen binding now seems less likely to be correct, but the question remains open. Recent work on the structure and spectra of haem compounds, including cobalt haemoglobin, suggests that molecular oxygen binds as a superoxide ion, and that many of Griffith's ideas are still relevant.

It was also in the early sixties that he became progressively more interested in the possible contributions that mathematicians could make to studies of the central nervous system. A few of his colleagues tried to dissuade him from moving into this field, but he persisted and was indeed to be followed by some of them. He left Philadelphia and returned to Cambridge to begin his work in earnest. First he studied the statistical properties of spontaneous impulse activity in cortical cells and the way in which activity in a neurone influences the excitability of an adjacent neurone. This work was closely tied to experimental observations.

At the same time he developed a field theory for predicting the overall be-

haviour of impulse activity in neural networks, which he described by local differential equations. He became interested in neuronal differentiation and suggested a molecular basis for the differences between classes of nerve cells. He considered that an alteration in the connexions between neurones is the most plausible theory for the neural basis of memory and turned to consider how such alterations could be initiated. In his "two gates" hypothesis he suggested that when two synaptically coupled cells fire together, their membranes become permeable to a chemical messenger substance which passes across the synapse from one cell to the other. He then went on to show how such a substance might act in order to change the functional connexions between the two cells. This was probably his most important contribution to brain theory. It created widespread interest although, because of technical difficulties, it remains to be tested. In 1967 he produced a short monograph entitled *A View of the Brain*, presenting, among other things, some controversial ideas about the establishment of connexions in the visual system, and 1971 saw the publication of his last book, completed during his long final illness, entitled *Mathematical Neurobiology*, in which he outlined the mathematical theory of neuronal networks, of action potentials and of information storage systems in general. It will be some time before the full implications of Griffith's original contributions to this subject are fully worked out.

No account of John Griffith's life would be complete without a reference to his deep passion for mathematical purity, his thoroughly original approach to any problem in which he was interested, and — behind a somewhat reserved exterior — a quiet shy smile that endeared him to his friends.

Announcements

International Meetings

July 2–14, **Padé Approximants and their Applications**, Canterbury (Dr A. K. Common, School of Mathematics, University of Kent, Canterbury, Kent).

July 3–4, **Advances in Applied Spectroscopy**, London (Conference Administrator, IPC Business and Industrial Training Limited, 161–166 Fleet Street, London EC4).

July 3–5, **Band Structure in Solids**, Exeter (Professor E. P. Wohlfarth, Department of Mathematics, Imperial College, London SW7).

July 3–5, **British Society for the History of Science Meeting**, Exeter (Assistant Secretary, British Society for the History

of Science, 47 Belgrave Square, London SW1).

July 3–7, **Models, Evaluations and Information Systems for Planners**, Cambridge (Richard Baxter, Centre for Land Use and Built Form Studies, 16 Brooklands Avenue, Cambridge CB2 2BB).

July 3–7, **Tropical Hygiene**, London (Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT).

July 3–7, **Sex Education**, Tel Aviv (Dr E. Chigier, PO Box 16271, Tel Aviv, Israel).

July 3–7, **Nuclear Magnetic Resonance Spectroscopy**, Guildford (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

July 3–8, **Chemistry in Evolution and Systematics**, Strasbourg (Dr J. B. Harborne, Phytochemical Unit, Botany Department, The University, Reading RG1 5AQ).

July 3–20, **Athens Ekistics Month**, Athens (P. Psomopoulos, Athens Centre of Ekistics, Box 471, Athens, Greece).

July 3–29, **Plasma Physics**, Les Houches (Ecole d'Eté de Physique Théorique, 74 Les Houches, Haute Savoie, France).

July 4, **The Measurement of Radiative Lifetimes of Atoms and Simple Molecules**, Oxford (The Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

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